농학박사학위논문

*Fusarium graminearum*의 cytochrome P450 효소들의 기능연구

Functional characterization of cytochrome P450 monooxygenases in the cereal head blight fungus *Fusarium graminearum*

2017년 8월

서울대학교 대학원

농생명공학부 식물미생물전공

신 지 영

Functional characterization of cytochrome P450 monooxygenases in the cereal head blight fungus *Fusarium graminearum*

A dissertation submitted in partial

fulfillment of the requirement for

the degree of

DOCTOR OF PHILOSOPHY

to the Faculty of

Department of Agricultural Biotechnology

at

SEOUL NATIONAL UNIVERSITY

By

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August, 2017

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이 논문을 농학석사학위논문으로 제출함
2017년 7월

서울대학교 대학원
농생명공학부 식물미생물학전공
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신 지 영의 박사학위논문을 인준함
2017년 6월

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A THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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UNDER THE DIRECTION OF
DR. YIN-WON LEE

SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL
OF SEOUL NATIONAL UNIVERSITY

BY
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MAJOR IN PLANT MICROBIOLOGY
DEPARTMENT OF AGRICULTURAL BIOTECHNOLOGY

JUNE 2017

APPROVED AS A QUALIFIED THESIS OF JIYOUNG SHIN
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY
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Abstract

Functional characterization of cytochrome P450 monooxygenases in the cereal head blight fungus *Fusarium graminearum*

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Fusarium graminearum is a prominent plant pathogenic fungus causing *Fusarium* head blight in major cereal crops worldwide. To understand the molecular mechanisms underlying fungal development and virulence, large collections of *F. graminearum* mutants have been constructed. Cytochrome P450 monooxygenases (P450s) are widely distributed in organisms and are involved in a diverse array of molecular/metabolic processes; however, no systematic functional analysis of P450s has been attempted in filamentous fungi. In this study, we constructed a genome-wide deletion mutant set covering 102 P450s and analyzed

these mutants for changes in 38 phenotypic categories, including fungal development, stress responses, and responses to several xenobiotics, to build a comprehensive phenotypic dataset. Most P450 mutants showing defective phenotypes were impaired in a single phenotypic trait, demonstrating that my mutant library is a good genetic resource for further fungal genetic studies. In particular, we identified novel P450s specifically involved in virulence (5) and both asexual (1) and sexual development (2). Most P450s seem to play redundant roles in the degradation of xenobiotics in *F. graminearum*. This study is the first phenome-based functional analysis of P450s, and it provides a valuable genetic resource for further basic and applied biological research in filamentous fungi and other plant pathogens.

Keywords: *Fusarium graminearum*, Cytochrome P450 monooxygenase, Mutant library, Sexual development, Virulence, Azole fungicide sensitivity, Xenobiotic induction

Student Number: 2012-23383

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INTRODUCTION

Systematic gene knockout mutant libraries and the corresponding phenotypic datasets provide powerful tools for studying the molecular mechanisms underlying various biological processes and for identifying molecular targets that can be utilized for medical or agricultural applications. Genome-wide sets of gene deletions in model yeasts have given rise to a new era in the genetics of eukaryotic cells (Winzeler et al., 1999; Kim et al., 2010). Despite decades of progress in the genetics of filamentous fungi, their significance to public health, food security, and ecosystems has been increasing worldwide (Fisher et al., 2012). However, no whole-gene deletion mutant library is available for filamentous fungi, and genome-wide functional genetic analyses have mainly focused on signaling mediators, such as transcriptional regulators (Colot et al., 2006; Son et al., 2011b; Jung et al., 2015), kinases (Wang et al., 2011; De Souza et al., 2013; Lee et al., 2016), and phosphatases (Yun et al., 2015).

Cytochrome P450 enzymes (P450s) are proteins belonging to a superfamily containing heme as a cofactor and are found in most organisms, including fungi (Nelson et al., 1996). P450s commonly function as monooxygenases that transfer molecular oxygen to substrates and mediate diverse chemical reactions (e.g., hydroxylation, dealkylation, epoxidation, deamination, desulfuration, dehalogenation, sulfoxidation, and N-oxide reduction) (Sono et al., 1996; Mansuy, 1998). Therefore, P450 catalysis participates in various important cellular/metabolic processes, including the biosynthesis of hormones and cell

structure, secondary metabolism, fatty acid metabolism, and the degradation of xenobiotics (toxins, carcinogens, and pollutants) (Werck-Reichhart and Feyereisen, 2000).

Approximately 0.3% of genes in the animal kingdom encode P450s (Ingelman-Sundberg, 2005). The human genome contains 57 P450-encoding genes, which are well-known for their drug-metabolizing activities. The rice and *Arabidopsis thaliana* genomes contain 437 (0.8%) and 276 (0.8%) genes encoding putative P450s (Park et al., 2008). Some of these enzymes are known to be involved in the biosynthesis of various secondary metabolites that function as chemical defenses against microbial pathogens and herbivores (Bolwell et al., 1994; Anzenbacher and Anzenbacherova, 2001).

There are considerable disparities in P450 gene numbers among fungal species, depending on their lifestyle. Only three (0.06%), ten (0.16%), and six (0.09%) P450s have been identified in the budding yeast *Saccharomyces cerevisiae* and the opportunistic fungal pathogens *Candida albicans* and *Cryptococcus neoformans*, respectively (McCullough et al., 1996; Sabiiti and May, 2012). However, plant pathogenic fungi tend to harbor more P450 genes than other fungal species. The rice blast fungus *Magnaporthe oryzae* exhibits 107 P450s (0.97%), and *Cryphonectria parasitica*, which causes chestnut blight, displays 121 P450s (1.08%). Several fungal P450s have been shown to play critical roles in primary and secondary metabolism and the degradation of xenobiotics (Črešnar and Petrič, 2011). However, the involvement of P450s in fungal development and other

functions, such as xenobiotic detoxification, has rarely been elucidated, and there has been no systematic approach to the investigation of P450 genes in filamentous fungi, including plant pathogens.

The ascomycete fungus *Fusarium graminearum* is an economically important plant pathogen that causes head blight in major cereal crops, such as wheat, barley, and rice (Leslie and Summerell, 2006). In addition to yield losses, this fungus contaminates grains with mycotoxins (deoxynivalenol and zearalenone), which pose a serious threat to human and animal health (Desjardins and Proctor, 2007). The genome of *F. graminearum* contains 119 P450 genes (0.9%), although only eight P450s have been functionally characterized in this fungus. Three P450s (*TRI1*, *TRI4*, and *TRI11*) are trichothecene biosynthetic genes (Brown et al., 2001; Kimura et al., 2007), and *Fg08079* and *CLM2* are required for the biosynthesis of butenolide (Harris et al., 2007) and culmorin (Bahadoor et al., 2016), respectively. Three other genes (*CYP51A*, *CYP51B*, and *CYP51C*) are paralogs of *CYP51*, which encodes an ergosterol biosynthetic enzyme (Liu et al., 2011; Fan et al., 2013).

In this study, I constructed a genome-wide P450 gene deletion mutant library for *F. graminearum*. Each mutant was analyzed in 23 phenotypic categories and under 15 xenobiotic induction conditions. My genetic resources and findings contribute to determining the functions of P450s in various cellular processes in filamentous fungi and in other lower eukaryotes.

MATERIALS AND METHODS

I. Fungal strains and media

The *F. graminearum* wild-type strain Z-3639 was used for generating deletion mutants (Bowden and Leslie, 1999). The wild-type and all transgenic strains were stored as mycelia and conidia in a 20% glycerol solution at -70 ℃. The culture media were prepared and used according to the *Fusarium* laboratory manual (Leslie and Summerell, 2006).

II. DNA extraction Southern blotting and PCR

Genomic DNA was isolated from freeze-dried mycelium powder as previously described (Leslie and Summerell, 2006). Standard techniques were used for restriction endonuclease digestion, agarose gel electrophoresis, and Southern blot analysis (Sambrook and Russell, 2001). The primers employed in this study were synthesized by an oligonucleotide synthesis facility (Neo Probe, Daejeon, Korea; Supporting information Table S6). General PCR was performed following the manufacturer's instructions (Takara Bio Inc., Otsu, Japan).

Table 1. . List and phenotypic summary of P450s in *F. graminearum.*

Locus ID	Gene name	Class	Best hit of Nelson's	Mycelia growth			Pigmentation	Sexual development				Conidiation	Toxin production		Virulence	Stress responses										Xenobiotics
				CM	PDA	MM		NP	PM	AF	AD		ZEA	DON		Osmotic	ROS	Fungicide	Cell wall	pH4	pH11	Heat	Cold	UV	Azole fungicides	
FGSG_00127.3	*Fg00127*	B-class P450	CYP645A1	4[a]	4[b]	4[c]	4[d]	4[e]	4[f]	4[g]	4[h]	4[i]	4[j]	4[j]	4[k]	4[l]	4[l]	4[l]	4[l]	4[l]	4[l]	4[l]	4[l]	4[l]	4[m]
FGSG_11585.3	*Fg11585*	B-class P450	CYP55A8	N/A[o]	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
FGSG_10960.3	*Fg10960*	B-class P450	N/M[p]	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_12284.3	*Fg12284*	Cytochrome P450	CYP587B1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
FGSG_03542.3	*Fg03542*	Cytochrome P450	CYP526B1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_14006.3	*Fg14006*	Cytochrome P450	CYP570C2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
FGSG_13739.3	*Fg13739*	Cytochrome P450	CYP631A1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_12371.3	*Fg12371*	Cytochrome P450	CYP639A1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
FGSG_01583.3	*Fg01583*	Cytochrome P450	CYP642A1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	5, cc[q]	3	4
FGSG_13796.3	*Fg13796*	Cytochrome P450	CYP644A1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_07859.3	*Fg07859*	Cytochrome P450	N/M	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_05334.3	*Fg05334*	Cytochrome P450	N/M	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_02668.3	*Fg02668*	Cytochrome P450	N/M	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	3	4
FGSG_09033.3	*Fg09033*	Cytochrome P450	N/M	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
FGSG_14007.3	*Fg14007*	Cytochrome P450	N/M	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_00863.3	*Fg00863*	Cytochrome P450	N/M	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_08377.3	*Fg08377*	E-class P450, group I	CYP544A3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4

FGSG_12283.3	*Fg12283*	E-class P450, group I	CYP587B1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
FGSG_02366.3	*Fg02366*	E-class P450, group I	CYP59B1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_03860.3	*Fg03860*	E-class P450, group I	CYP526B1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_09916.3	*Fg09916*	E-class P450, group I	CYP534C1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_01972.3	*Fg01972*	E-class P450, group I	CYP505A7	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	2
FGSG_07596.3	*Fg07596*	E-class P450, group I	CYP505C2	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_11303.3	*Fg11303*	E-class P450, group I	CYP547B1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_01048.3	*Fg01048*	E-class P450, group I	CYP617A1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	3	4
FGSG_10461.3	*Fg10461*	E-class P450, group I	CYP617C1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_07977.3	*Fg07977*	E-class P450, group I	CYP5080A4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_02458.3	*Fg02458*	E-class P450, group I	CYP528A2	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_09351.3	*Fg09351*	E-class P450, group I	CYP531D1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_01745.3	*Fg01745*	E-class P450, group I	CYP532A3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	3	4
FGSG_12737.3	*Fg12737*	E-class P450, group I	CYP537A2	4	4	4	4	4	4	4	4	4	4	4	2	4	4	4	4	4	4	4	4	4	4	4
FGSG_11655.3	*Fg11655*	E-class P450, group I	CYP548B1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_01739.3	*Fg01739*	E-class P450, group I	CYP551B1	3	3	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	3	4
FGSG_11038.3	*Fg11038*	E-class P450, group I	CYP552B3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_02344.3	*Fg02344*	E-class P450, group I	CYP552C2	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_10695.3	*Fg10695*	E-class P450, group I	CYP567B1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_02419.3	*Fg02419*	E-class P450, group I	CYP570C1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	3	4

FGSG_02929.3	*Fg02929*	E-class P450, group I	CYP570C1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	3	4
FGSG_11498.3	*Fg11498*	E-class P450, group I	CYP570D1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_12534.3	*Fg12534*	E-class P450, group I	CYP573A1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_13176.3	*Fg13176*	E-class P450, group I	CYP577A2	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_07804.3	*Fg07804*	E-class P450, group I	CYP627A1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_03086.3	*Fg03086*	E-class P450, group I	CYP628A1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
FGSG_11424.3	*Fg11424*	E-class P450, group I	CYP629A1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_00012.3	*Fg00012*	E-class P450, group I	CYP630A1	4	4	4	4	4	4	4	4	4	4	4	2	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_08412.3	*Fg08412*	E-class P450, group I	CYP630B1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_03264.3	*Fg03264*	E-class P450, group I	CYP632A1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_02367.3	*Fg02367*	E-class P450, group I	CYP643A1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_01722.3	*Fg01722*	E-class P450, group I	CYP643A2	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	3	4
FGSG_12599.3	*Fg12599*	E-class P450, group I	CYP643B1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_03535.3	*TRI4*	E-class P450, group I	CYP58A3	4	4	4	4	4	4	4	4	4	4	4	0	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_03741.3	*Fg03741*	E-class P450, group I	CYP504A5	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_06447.3	*Fg06447*	E-class P450, group I	CYP504B3	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
FGSG_10571.3	*Fg10571*	E-class P450, group I	CYP5065A1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
FGSG_11389.3	*Fg11389*	E-class P450, group I	CYP530A2	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_09086.3	*Fg09086*	E-class P450, group I	CYP619A1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
FGSG_12568.3	*Fg12568*	E-class P450, group I	CYP620A1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4

FGSG_03700.3	*Fg03700*	E-class P450, group I	CYP620B1	4	4	4	4	4	4	4	4	4	4	4	2	4	4	4	4	4	4	4	4	4	4	4
FGSG_00007.3	*CLM2*	E-class P450, group I	CYP620C1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_05113.3	*Fg05113*	E-class P450, group I	CYP621A1	3	3	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	3	4
FGSG_08809.3	*Fg08809*	E-class P450, group I	CYP580A1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_02113.3	*Fg02113*	E-class P450, group I	CYP614A1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
FGSG_08183.3	*Fg08183*	E-class P450, group I	CYP615A1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_09671.3	*Fg09671*	E-class P450, group I	CYP616A1	4	4	4	4	1	1	0	0	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_02371.3	*Fg02371*	E-class P450, group I	CYP624A1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_10991.3	*Fg10991*	E-class P450, group I	CYP625A1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_11465.3	*Fg11465*	E-class P450, group I	CYP626A1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_10629.3	*Fg10629*	E-class P450, group IV	CYP526F1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_02138.3	*Fg02138*	E-class P450, group IV	CYP540B2	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_03548.3	*Fg03548*	E-class P450, group IV	CYP540B3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_02118.3	*Fg02118*	E-class P450, group IV	CYP617B1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_12342.3	*Fg12342*	E-class P450, group IV	CYP618A1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
FGSG_01767.3	*Fg01767*	E-class P450, group IV	CYP527E1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	3	4
FGSG_08186.3	*Fg08186*	E-class P450, group IV	CYP552C3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_04590.3	*Fg04590*	E-class P450, group IV	CYP566B1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
FGSG_01959.3	*Fg01959*	E-class P450, group IV	CYP61A1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
FGSG_03686.3	*Fg03686*	E-class P450, group IV	CYP61Ax	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4

FGSG_05960.3	*Fg05960*	E-class P450, group IV	CYP550B1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4, cc	4	4, cc	4
FGSG_03796.3	*Fg03796*	E-class P450, group IV	CYP633A1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_08207.3	*Fg08207*	E-class P450, group IV	CYP633B1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_08191.3	*Fg08191*	E-class P450, group IV	CYP635A1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
FGSG_03498.3	*Fg03498*	E-class P450, group IV	CYP637A1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	3	4
FGSG_02114.3	*Fg02114*	E-class P450, group IV	CYP638A1	3	3	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	2	4
FGSG_12372.3	*Fg12372*	E-class P450, group IV	CYP639A1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
FGSG_10910.3	*Fg10910*	E-class P450, group IV	CYP641A1	3	3	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_01000.3	*CYP51B*	E-class P450, group IV	CYP51F1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_04092.3	*CYP51A*	E-class P450, group IV	CYP51F2	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	1	4
FGSG_11024.3	*CYP51C*	E-class P450, group IV	CYP51F3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_11536.3	*Fg11536*	E-class P450, group IV	CYP54C1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_01868.3	*Fg01868*	E-class P450, group IV	CYP602B1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_11282.3	*Fg11282*	E-class P450, group IV	CYP606B1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_08187.3	*Fg08187*	E-class P450, group IV	CYP622A1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_02117.3	*Fg02117*	E-class P450, group IV	CYP623A1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_09195.3	*Fg09195*	E-class P450, group IV	CYP623B1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_00071.3	*TRI1*	E-class P450, group IV	CYP68C2	4	4	4	4	4	4	4	4	4	4	0	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_02672.3	*Fg02672*	E-class P450, group IV	CYP68J1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	3	4
FGSG_04717.3	*Fg04717*	E-class P450, group IV	CYP68J2	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4

FGSG_11002.3	*Fg11002*	E-class P450, group IV	CYP68K1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_01786.3	*Fg01786*	E-class P450, group IV	CYP506A2	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_02872.3	*Fg02872*	E-class P450, group IV	CYP634A1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_02111.3	*Fg02111*	E-class P450, group IV	CYP636A1	4	4	4	4	4	4	4	4	4	4	4	4	2	4	4	4	4	4	4	4	4	4	4	4
FGSG_11108.3	*Fg11108*	E-class P450, group IV	CYP640A1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_13797.3	*Fg13797*	E-class P450, group IV	CYP644A1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_01284.3	*Fg01284*	P450, CYP52	CYP539A3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_08005.3	*Fg08005*	P450, CYP52	CYP539E1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_08320.3	*Fg08320*	P450, CYP52	CYP584D1	4	4	4	4	5	2	0	0	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_13459.3	*Fg13459*	Pisatin demethylase-like	CYP5078A2	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_03260.3	*Fg03260*	Pisatin demethylase-like	CYP5080A1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_08079.3	*Fg08079*	Pisatin demethylase-like	CYP53A6	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	3	4
FGSG_13445.3	*Fg13445*	Pisatin demethylase-like	CYP53A7	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_10451.3	*Fg10451*	Pisatin demethylase-like	CYP53A8	3	3	3	4	4	4	4	4	4	4	4	2	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_02982.3	*Fg02982*	Pisatin demethylase-like	CYP531C1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_06068.3	*Fg06068*	Pisatin demethylase-like	CYP548A3	4	4	4	4	4	4	4	4	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_01740.3	*Fg01740*	Pisatin demethylase-like	CYP561D1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	3	4
FGSG_05806.3	*Fg05806*	Pisatin demethylase-like	CYP62B1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_03540.3	*TRI11*	Pisatin demethylase-like	CYP65A2	4	4	4	4	4	4	4	4	4	4	0	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_07765.3	*Fg07765*	Pisatin demethylase-like	CYP65Q1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4

FGSG_03191.3	*Fg03191*	Pisatin demethylase-like	CYP65R1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
FGSG_08023.3	*Fg08023*	Pisatin demethylase-like	CYP65S1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

[a] Percentage of average radial growth on potato complete media (CM) of deletion mutants compared to wild-type strain was scored (5, more than wild-type strain; 4, 91-100%; 3, 61-90%; 2, 31-60 %; 1, 0-30 %; 0, 0 %).

[b] Percentage of average radial growth on potato dextrose agar media (PDA) of deletion mutants compared to wild-type strain was scored (5, more than wild-type strain; 4, 91-100%; 3, 61-90%; 2, 31-60 %; 1, 0-30 %; 0, 0 %).

[c] Percentage of average radial growth on minimal media (MM) of deletion mutants compared to wild-type strain was scored (5, more than wild-type strain; 4, 91-100%; 3, 61-90%; 2, 31-60 %; 1, 0-30 %; 0, 0 %).

[d] Pigmentation representing aurofusarin accumulation after 5 days of growth on PDA was scored by comparing with wild-type strain (5, more than wild-type strain; 4, similar to wild-type strain; 2, less than wild-type strain; 0, no pigmentation).

[e] Number of perithecium (NP) was scored from “0” to “5” (1, less than 25% of the wild-type strain; 2, 25-50% less; 3, 50-75% less; 4, 75-100% less; 5, more than wild-type strain; 0, no perithecium).

[f] Perithecia maturation (PM) was determined by size compared to wild-type strain (4, normal; 3, 1-2 day delayed; 2, 3-7 days delayed; 1, more than 7 days delayed maturation).

[g] Ascospore formation (AF) represents the existence and morphology of ascospores (4, normal ascospores; 2, abnormal shape of ascospore; 0, no observable ascospores).

[h] Ascospore discharge (AD) was determined by discharged number of ascospores compared to wild-type strain (4, normal; 2, reduced; 0, no discharge).

[i] Conidiation represents conidia production of mutants compared to wild-type strain (4, similar number to wild-type strain; 3, approximately 10-fold reduction; 2, approximately 100-fold reduction; 1, approximately 1000-fold reduction; 0, no visible conidia production).

[j] Toxin production (ZEA, zearalenone; DON, deoxynivalenol) was quantified compared to wild-type strain (6, overproduction; 4, similar to wild-type strain; 2, reduced production; 0, no detectable production).

[k] Virulence on wheat heads was scored (4, normal; 0, markedly reduced virulence compared to wild-type strain).

[l] Radial growth of mutants on each stress-inducing media was evaluated compared to growth on mock complete media (CM) (2, susceptible; 4, normal; 6, resistant).

[m] Radial growth of mutants on each xenobiontics media was evaluated compared to growth on mock complete media (CM) (2, susceptible; 4, normal; 6, resistant).

[o] N/A: not applicable.

[p] N/M: not matched.

[q] Color change of pigment (cc)

III. Generation of P450 gene deletion mutants

DNA cassettes for targeted gene deletion were generated using the double-joint (DJ) PCR and split marker recombination methods (Catlett et al., 2003; Yu et al., 2004). The *GEN* was amplified from pII99 (Namiki et al., 2001), and the 5′ and 3′ regions of the target gene were amplified from Z-3639. Three amplicons (5′ region, *GEN*, and 3′ region) were fused via DJ PCR using previously described conditions (Yu et al., 2004), and fungal transformation was performed as previously described (Son et al., 2011a). At least three independent transformants were isolated for each gene deletion via co-dominant PCR screening. To exclude ectopic mutants, (P450 name)-5F and Gen-with 5F primers were used for PCR screening. To confirm a single copy integration of the mutant allele, Southern hybridization was performed (Supporting information Fig. S1).

IV. Asexual and sexual development

In most cases, two independent mutants for each gene deletion were used for phenotyping with three replications. Colony morphology and radial growth were assayed with complete medium (CM), potato dextrose agar (PDA) and minimal medium (MM) at 25 ℃ for three and five days after inoculation. The pigmentation of the bottom of the PDA plates (representing aurofusarin accumulation) after being cultured for five days was compared with the wild-type strain, and the intensity of the red color was scored (0-6) with the naked eye.

Conidium production was measured by counting the number of conidia with a

hemocytometer (Superior, Marienfeld, Germany) after culturing a culture plug of CM in 5 ml of carboxymethylcellulose medium (CMC) for five days at 25 ℃ on a rotary shaker (150 rpm). For the observation of conidium morphology, the conidia produced by each strain on yeast malt agar (YMA) were harvested, and differential interference contrast (DIC) images were obtained using a DE/Axio Imager A1 microscope (Carl Zeiss, Oberkochen, Germany).

For sexual development, each mutant was incubated on carrot agar at 25 ℃ for 5 days (Leslie and Summerell, 2006). Mycelia grown on carrot agar were mock fertilized with 700 μl of a 2.5% Tween 60 solution to induce sexual reproduction and were then incubated under a near-UV light (wavelength: 365 nm, HKiv Import & Export Co., Ltd., Xiamen, China) at 25 ℃. Seven days after sexual induction, the maturation of perithecia was determined by measuring their size.

V. Virulence test and mycotoxin analysis

For the virulence test, the point inoculation method was employed, as previously described (Lee et al., 2009). The conidia of each strain were harvested from CMC and adjusted to 1×10^5 conidia/ml. Thereafter, 10 μl of each conidial suspension was injected into a center spikelet of a wheat head (cultivar Eunpamil) at mid-anthesis. The wheat plants were subsequently incubated in a humid chamber for 3 days and grown in a greenhouse for an additional 18 days. The first virulence test was performed

using two independent strains for each gene deletion and five wheat heads for each strain. Mutants showing typical FHB symptoms were excluded from the second virulence test. Infected wheat heads of reduced virulence ($p < 0.05$, *t* test) were imaged, and spikelets with head blight symptoms of ten wheat heads were counted in the second virulence test.

To determine the effect of P450 gene deletion on mycotoxin production, fungal strains were grown on 30 g of rice substrate for three weeks at 25 ℃ in the dark. Rice cultures were harvested, and mycotoxins were extracted as previously described (Seo et al., 1996). A portion of each extract was analyzed using TLC to estimate the amounts of DON and zearalenone. An ELISA test kit (Veratox® , Neogen Corp., Lansing, MI, USA) was applied for exact quantifications of DON and zearalenone in accordance with the manufacturer's instructions. The experiments were performed using two independent strains with three biological replicates for each gene deletion.

VI. Stress and azole fungicide sensitivity screening

A mycelial plug (5 mm in diameter) was collected from the periphery of a 3-day-old colony and placed on a CM plate supplemented with various stress agents as previously described (Son et al., 2011b). The tested stress conditions included oxidative stress (5 mM hydrogen peroxide and 0.1 mM menadione), osmotic stress (1 M NaCl, 1 M KCl, 1.5 M sorbitol, and 6 mM $FeSO_4$), pH stress (pH = 4 and pH

= 11), cell wall stress (60 mg/L Congo Red and 5 mg/L sodium dodecyl sulfate), inhibition of DNA synthesis (8.6 mg/L fungicide iprodione), inhibition of the mitogen activated protein kinase pathway (0.023 mg/L fungicide fludioxonil), inhibition of meiosis and intracellular transportation (0.65 mg/L fungicide benomyl), UV stress (365 nm; HKiv Import & Export Co.), and treatment with azole fungicides (0.025 mg/L tebuconazole, 0.05 mg/L difenoconazole, 0.1 mg/L epoxiconazole, 0.05 mg/L propiconazole, and 0.01 mg/L prochloraz) (Liu et al., 2011).

VII. Assay for xenobiotic induction

A total of 15 xenobiotics, including aliphatic (176 mM n-dodecane, 22 mM 1-dodecanol, and 10 mM levulinic acid), alicyclic (48.2 mM cyclohexanone), aromatic (15 mM methyl benzoate, 0.14 mM 4-nitrophenol, 0.53 mM phenoxyacetic acid, and 0.72 mM resorcinol), polyaromatic (0.32 mM benzo(a)pyrene, 0.15 mM 3-methyl cholanthrene, 0.14 mM 1-naphthol, 0.62 mM naphthalene, and 0.62 mM phenanthrene), and alkyl-substituted aromatic (0.06 mM sodium dodecylbenzenesulfonate and 0.1 mM 4-octylphenol) compounds, were tested for P450 transcriptional induction in liquid CM cultures. Xenobiotic chemicals of analytical grade were used, which were purchased from different commercial sources. CM cultures grown for 24 h at 25 ℃ on a rotary shaker (150 rpm) were spiked with individual chemicals, and mycelia were harvested after 24 h (Doddapaneni et al., 2005). The details of the tested xenobiotics and the molar

concentrations used are given in Supporting information Table S4. A parallel control culture treated with an equal volume of the solvent [dimethyl sulfoxide (DMSO) for solids, water for liquids] was also included.

VIII. Quantitative real time (qRT)-PCR

The total RNAs of the wild-type and P450 mutant strains were extracted from mycelia using the Easy-Spin Total RNA Extraction kit (iNtRON Biotech, Seongnam, Korea). First-strand cDNA was synthesized with the SuperScript III First-Strand Synthesis System (Invitrogen, Carlsbad, CA, USA). qRT-PCR was performed using iQ SYBR Green Master Mix (Bio-Rad, Hercules, CA, USA) and the 7500 real-time PCR system (Applied Biosystems, Foster City, CA, USA). For normalization, the endogenous housekeeping gene cyclophilin (*CYP1*; FGSG_07439.3) was used (Lin et al., 2011). The threshold cycle (ΔC_T) value of gene expression was subtracted from the ΔC_T value of each sample to obtain a $\Delta\Delta C_T$ value. The transcript level relative to the calibrator was expressed as $2^{-\Delta\Delta CT}$ (Livak and Schmittgen, 2001). The qRT-PCR assays were repeated three times with two biological replicates.

RESULTS

I. Characterization and deletion of putative P450 genes in *F. graminearum*

To identify *F. graminearum* P450 genes, I employed the previously constructed Fungal Cytochrome P450 Database (Park et al., 2008). The *F. graminearum* genome contains 119 putative P450 genes, which cover 0.9% of the total genes that are similar to other plant pathogenic fungi (Supporting information Table S1). These P450s were classified into six classes based on InterPro terms (Fig. 1A). Approximately 70% of the P450s (87) were included in the "E-class P450" category, and 13 P450s were grouped into the "Pisatin demethylase" category. The P450 enzymes were also grouped into families based on amino acid sequence similarity (Supporting information Table S1).

As little is known about the genetic requirements for P450s in fungal development, I analyzed the profiles of transcript levels during sexual and asexual development (Fig. 2). RNA-seq results were obtained from previous studies (Sikhakolli et al., 2012; Son et al., 2013; Son et al., 2016) and visualized using ClustVis (Metsalu and Vilo, 2015). Based on their expression patterns, P450s were clustered into sexually (A group) and asexually (B group) induced gene groups. Intriguingly, group A genes were specifically induced during sexual reproduction but repressed during asexual reproduction, while the opposite was true for group B genes, demonstrating that different P450-mediated molecular processes are

involved in fungal reproduction processes in *F. graminearum*.

To examine the biological functions of the P450s, I attempted to generate deletion mutants for all of the P450 genes. Using the split-marker homologous recombination method, I successfully replaced 102 of the P450 genes with a geneticin resistance gene cassette (*GEN*). The exact genetic manipulations of the selected the geneticin resistance mutants were confirmed via co-dominant PCR screening, followed by Southern hybridization with a *GEN* probe (Supporting information Fig. S1). I failed to delete 17 genes in more than three independent trials, possibly due to lethality. At least two independent deletion mutants of each P450 gene were characterized for their phenotypes in terms of mycelial growth, pigmentation, conidiation, sexual development, virulence on wheat heads, mycotoxin production, responses to various stresses, fungicides (including azole fungicides), and several types of xenobiotic induction (Supporting information Table S1).

II. Effects of P450 mutation on vegetative growth and conidiogenesis

I compared the vegetative growth and pigmentation of each P450 mutant in nutrient-rich media (CM, complete medium; PDA, potato dextrose agar medium) and nutrient-starved medium (MM, minimal medium). Most of the P450 mutants displayed similar phenotypes for mycelial growth under the tested conditions, with the exception of five mutants (Supporting information Table S1 and Fig. 3). The

growth rates of these five mutants (Δ*Fg02114*, Δ*Fg01739*, Δ*Fg05113*, Δ*Fg10910*, and Δ*Fg10451*) were slightly reduced compared with the wild-type strain (Fig. 3); however, they showed no difference in hyphal morphology.

Only one mutant was severely defective in asexual reproduction. The conidial production of the Δ*Fg06068* strains in carboxymethyl cellulose medium (CMC) was significantly reduced compared with the wild-type strain (Fig. 4A). Moreover, red pigment production during conidiation was mostly abolished in Δ*Fg06068*, whereas the mutant normally accumulated red pigments during vegetative growth on PDA, demonstrating that the deletion of *Fg06068* led to malfunctions of several important cellular processes during conidiation (Supporting information Table S1 and Fig. 4B).

To determine how deletion of the *Fg06068* gene affects conidiation, microscopic observations were performed. The *F. graminearum* wild-type strain initially produced phialides from the hyphae, and mature phialides continuously produced conidia (Fig. 4C). Although some phialides were observable in Δ*Fg06068*, the number of phialides was markedly reduced compared with the wild type, and most phialide cells were collapsed during conidiogenesis (Fig. 4C). However, the conidium morphologies of the Δ*Fg06068* and wild-type strains were indistinguishable.

To characterize the mechanistic roles of *Fg06068*, I analyzed the transcript levels of *Fg06068* and representative conidiation marker genes during conidiation.

The transcription of *Fg06068* was markedly induced after conidium induction in the wild-type strain (Fig. 4D). When I compared the transcript levels of representative conidiation-related genes between the wild-type and Δ*Fg06068* mutant strains (Ohara et al., 2004; Lysøe et al., 2011; Zheng et al., 2012), the transcript levels of all six genes (*STUA*, *HTF1*, *REN1*, *FLBC*, *ABAA*, and *WETA*) were observed to be significantly decreased in the Δ*Fg06068* mutants compared with the wild-type strain (Fig. 4D). Considering these findings together, I concluded that asexually induced *Fg06068* is required for phialide formation and function in *F. graminearum* and possibly participates in the regulation of the genetic pathway of conidiogenesis.

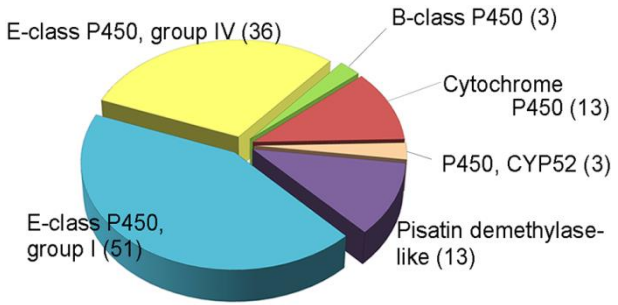
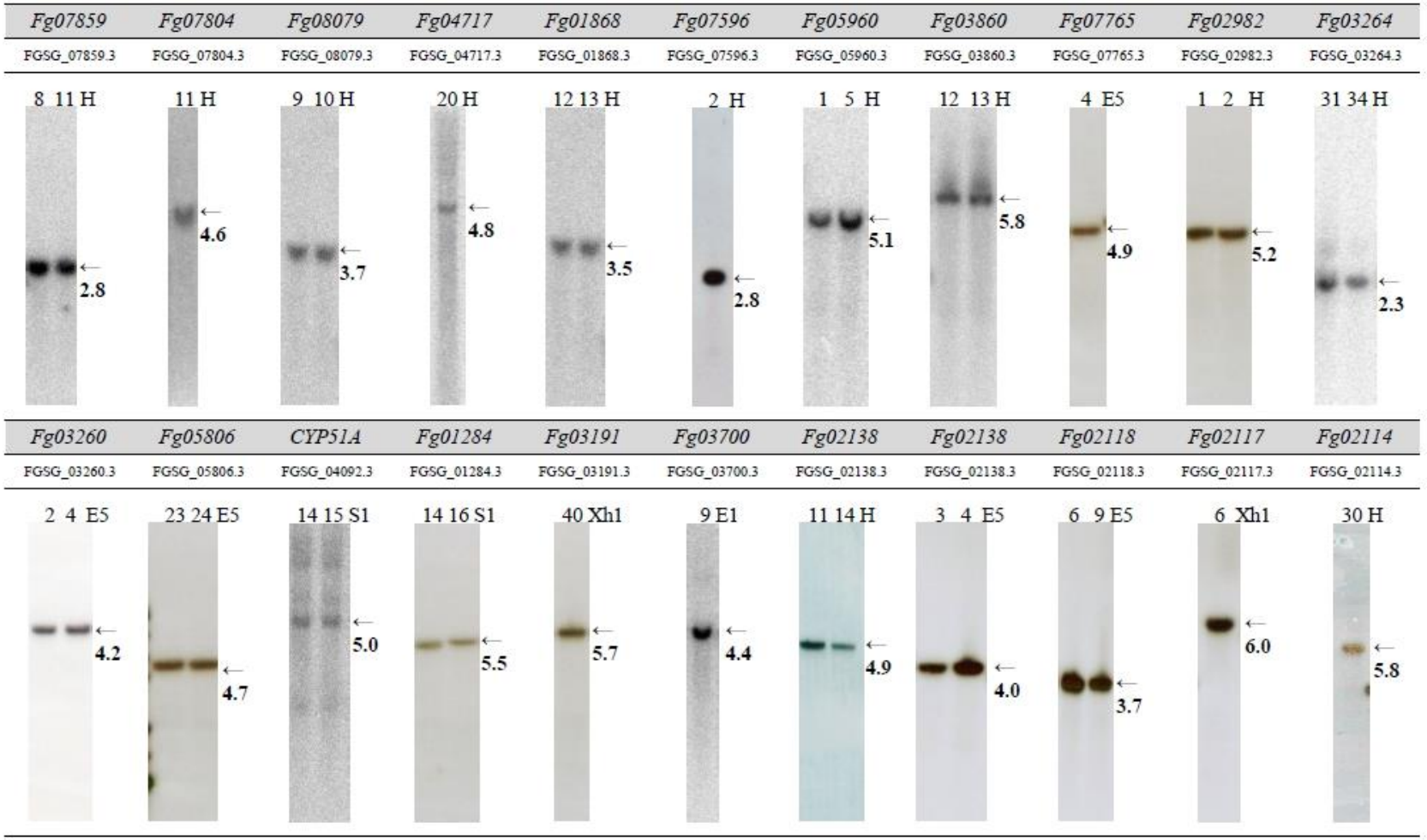
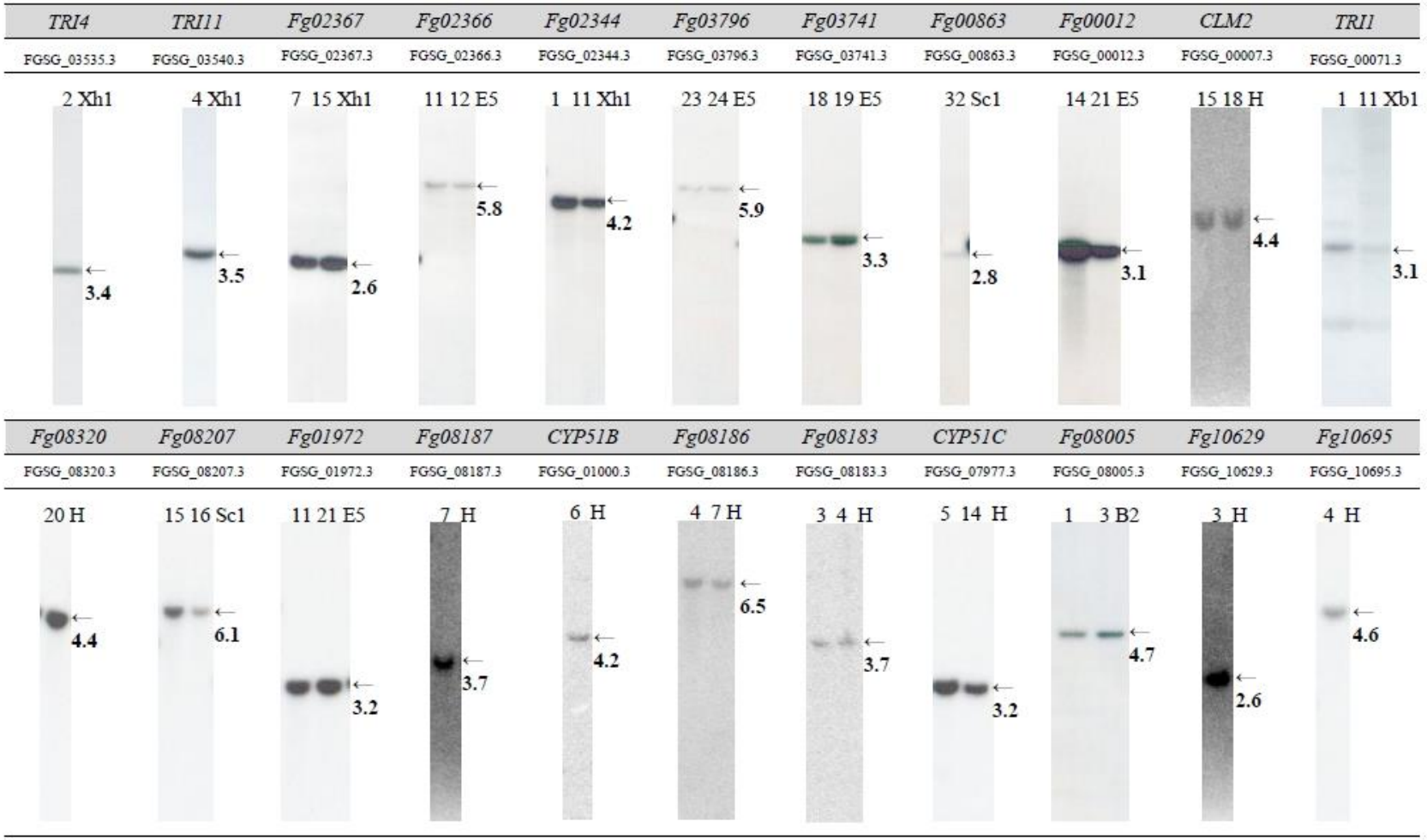


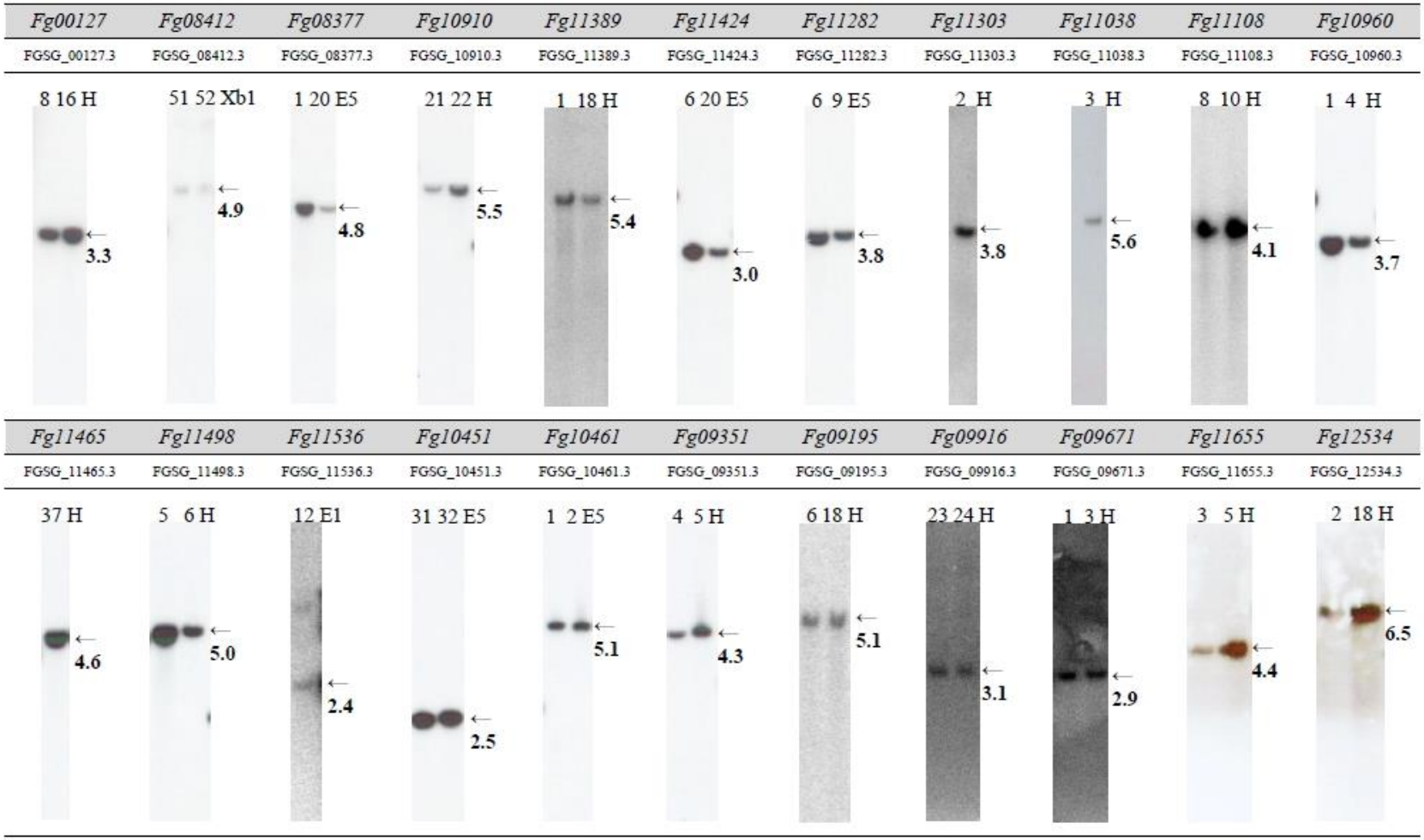
FIG 1. Classification of putative P450s in *F. graminearum*. The total P450s were categorized into six classes based on InterPro terms.


Fg07859
FGSG_07859.3
8 11 H
2.8
Fg07804
FGSG_07804.3
11 H
4.6
Fg08079
FGSG_08079.3
9 10 H
3.7
Fg04717
FGSG_04717.3
20 H
4.8
Fg01868
FGSG_01868.3
12 13 H
3.5
Fg07596
FGSG_07596.3
2 H
2.8
Fg05960
FGSG_05960.3
1 5 H
5.1
Fg03860
FGSG_03860.3
12 13 H
5.8
Fg07765
FGSG_07765.3
4 E5
4.9
Fg02982
FGSG_02982.3
1 2 H
5.2
Fg03264
FGSG_03264.3
31 34 H
2.3
Fg03260
FGSG_03260.3
2 4 E5
4.2
Fg05806
FGSG_05806.3
23 24 E5
4.7
CYP51A
FGSG_04092.3
14 15 S1
5.0
Fg01284
FGSG_01284.3
14 16 S1
5.5
Fg03191
FGSG_03191.3
40 Xh1
5.7
Fg03700
FGSG_03700.3
9 E1
4.4
Fg02138
FGSG_02138.3
11 14 H
4.9
Fg02138
FGSG_02138.3
3 4 E5
4.0
Fg02118
FGSG_02118.3
6 9 E5
3.7
Fg02117
FGSG_02117.3
6 Xh1
6.0
Fg02114
FGSG_02114.3
30 H
5.8

Fg02111	*Fg02458*	*Fg05334*	*Fg02672*	*Fg02668*	*Fg02929*	*Fg02872*	*Fg03548*	*Fg03542*	*Fg03489*	*Fg06068*
FGSG_02111.3	FGSG_02458.3	FGSG_05334.3	FGSG_02672.3	FGSG_02668.3	FGSG_02929.3	FGSG_02872.3	FGSG_03548.3	FGSG_03542.3	FGSG_03498.3	FGSG_06068.3
5 10 H	6 7 H	2 5 H	36 37 H	1 B2	28 E1	2 H	6 H	5 7 H	6 7 H	5 7 H
4.2	3.6	2.8	9.0 6.7	3.5	3.7	3.1	3.1	3.9	6.1	3.3

Fg01786	*Fg01767*	*Fg01745*	*Fg01740*	*Fg01739*	*Fg01722*	*Fg05113*	*Fg01583*	*Fg01048*	*Fg02419*	*Fg02371*
FGSG_01786.3	FGSG_01767.3	FGSG_01745.3	FGSG_01740.3	FGSG_01739.3	FGSG_01722.3	FGSG_05113.3	FGSG_01583.3	FGSG_01048.3	FGSG_02419.3	FGSG_02371.3
8 10 H	14 16 H	16 E5	6 9 H	2 24 H	9 11 H	2 18 E5	9 11 H	13 H	31 H	12 E5
6.1	6.6	4.9	6.1	4.0	3.8	3.6	2.4	3.2	3.0	4.2


TRI4
FGSG_03535.3
2 Xh1
3.4
TRI11
FGSG_03540.3
4 Xh1
3.5
Fg02367
FGSG_02367.3
7 15 Xh1
2.6
Fg02366
FGSG_02366.3
11 12 E5
5.8
Fg02344
FGSG_02344.3
1 11 Xh1
4.2
Fg03796
FGSG_03796.3
23 24 E5
5.9
Fg03741
FGSG_03741.3
18 19 E5
3.3
Fg00863
FGSG_00863.3
32 Sc1
2.8
Fg00012
FGSG_00012.3
14 21 E5
3.1
CLM2
FGSG_00007.3
15 18 H
4.4
TRI1
FGSG_00071.3
1 11 Xb1
3.1
Fg08320
FGSG_08320.3
20 H
4.4
Fg08207
FGSG_08207.3
15 16 Sc1
6.1
Fg01972
FGSG_01972.3
11 21 E5
3.2
Fg08187
FGSG_08187.3
7 H
3.7
CYP51B
FGSG_01000.3
6 H
4.2
Fg08186
FGSG_08186.3
4 7 H
6.5
Fg08183
FGSG_08183.3
3 4 H
3.7
CYP51C
FGSG_07977.3
5 14 H
3.2
Fg08005
FGSG_08005.3
1 3 B2
4.7
Fg10629
FGSG_10629.3
3 H
2.6
Fg10695
FGSG_10695.3
4 H
4.6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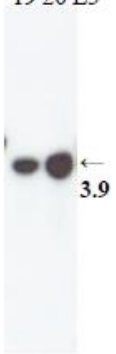
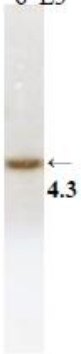
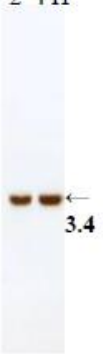
FGSG_00127.3
8 16 H
3.3
Fg08412
FGSG_08412.3
51 52 Xb1
4.9
Fg08377
FGSG_08377.3
1 20 E5
4.8
Fg10910
FGSG_10910.3
21 22 H
5.5
Fg11389
FGSG_11389.3
1 18 H
5.4
Fg11424
FGSG_11424.3
6 20 E5
3.0
Fg11282
FGSG_11282.3
6 9 E5
3.8
Fg11303
FGSG_11303.3
2 H
3.8
Fg11038
FGSG_11038.3
3 H
5.6
Fg11108
FGSG_11108.3
8 10 H
4.1
Fg10960
FGSG_10960.3
1 4 H
3.7
Fg11465
FGSG_11465.3
37 H
4.6
Fg11498
FGSG_11498.3
5 6 H
5.0
Fg11536
FGSG_11536.3
12 E1
2.4
Fg10451
FGSG_10451.3
31 32 E5
2.5
Fg10461
FGSG_10461.3
1 2 E5
5.1
Fg09351
FGSG_09351.3
4 5 H
4.3
Fg09195
FGSG_09195.3
6 18 H
5.1
Fg09916
FGSG_09916.3
23 24 H
3.1
Fg09671
FGSG_09671.3
1 3 H
2.9
Fg11655
FGSG_11655.3
3 5 H
4.4
Fg12534
FGSG_12534.3
2 18 H
6.5

Fg10991	*Fg11002*	*Fg11024*	*Fg12568*	*Fg08809*	*Fg13176*	*Fg12737*	*Fg12599*	*Fg13445*	*Fg13459*	*Fg14007*
FGSG_10991.3	FGSG_11002.3	FGSG_11024.3	FGSG_12568.3	FGSG_08809.3	FGSG_13176.3	FGSG_12737.3	FGSG_12599.3	FGSG_13445.3	FGSG_13459.3	FGSG_14007.3
6 13 E5	19 20 E5	12 13 H	6 E5	13 16 E5	5 22 H	13 E1	2 4 H	7 14 Xh1	3 H	2 E5
										
4.6	3.9	6.0	4.3	4.3	5.7	6.4	3.4	5.2	4.9	4.8

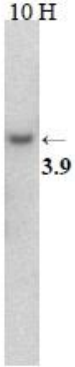
Fg13739	*Fg13796*	*Fg13797*
FGSG_13739.3	FGSG_13796.3	FGSG_13797.3
10 H	4 H	18 E5
		
3.9	2.5	4.4

FIG 2. Confirmation of P450 deletion mutants by Southern blotting. The restriction enzymes used for each blot and the size of the DNA standards (kb) are indicated on the right of each blot. H, HindIII; E1, EcoRI, E5, EcoRV; B, BglII; S, SalI; Sc, SacI; Nd, NdeI; Nr, NruI; B1, BamHI; Xh, XhoI; Xb, XbaI; P1, PstI; C1, ClaI; SS1, SspI; K1, KpnI

III. Sexual development

As half of the P450s were upregulated during several steps of sexual reproduction (Fig. 2), I next sought to identify the P450s involved in these processes. However, most of the P450 deletion mutants appeared normal in the production of perithecia and sexual spores (ascospores); only two P450 mutants (Δ*Fg08320* and Δ*Fg09671*) exhibited defects in sexual reproduction (Fig. 5). Δ*Fg09671* rarely produced perithecium initials, which did not mature further (Fig. 5A). While mycelial growth was mostly suppressed during sexual development in the wild-type strain, aerial mycelia grew upward and covered cultures of Δ*Fg09671*. The transcript levels of *Fg09671* were significantly induced until 3 days after sexual induction and declined as the perithecia matured, suggesting that *Fg09671* is required for the initial stages of perithecium development in *F. graminearum* (Fig. 5B).

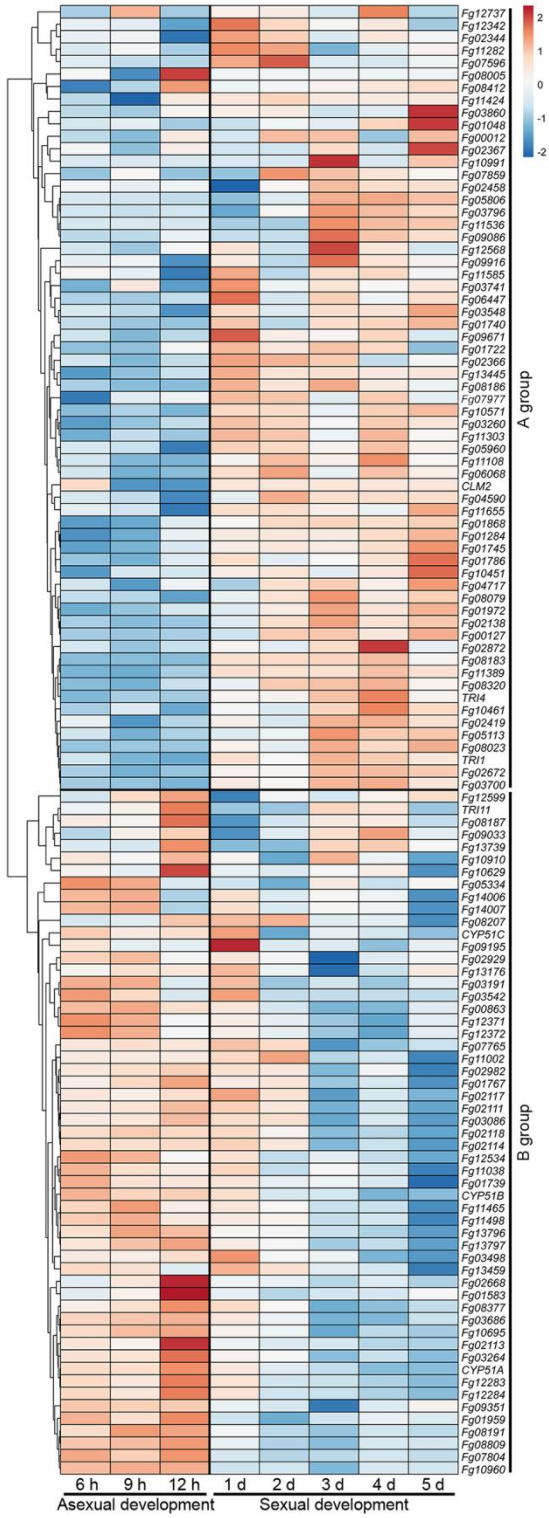
Deletion mutants of *Fg08320* produced smaller perithecia than the wild-type strain (Fig. 5A). Two days after sexual induction, both the wild-type and Δ*Fg08320* strains produced small perithecia with similar numbers (Fig. 5C and D). After 3 days of incubation, some of the small perithecia were selectively matured in the wild-type strain, and the others remained immature. However, Δ*Fg08320* produced a greater number of small perithecia than the wild type, and none of them matured (Fig. 5C and D). Finally, the average perithecium diameter produced by Δ*Fg08320* was approximately 110 μm, which was nearly 2-fold smaller than the perithecia produced by the wild-type strain ($p < 0.05$) (Fig. 5D). *Fg08320* transcripts accumulated dramatically 3 days after sexual induction and decreased as the

perithecia matured, similar to *Fg09671* (Fig. 5B). Thus, both P450 genes are specifically involved in perithecium development and/or ascospore formation in *F. graminearum*. In particular, *Fg08320* plays roles in the determination of perithecium numbers and selective perithecium maturation.

IV. Virulence and mycotoxin production

To evaluate the virulence of the P450 mutants, a conidial suspension of each strain was point-inoculated onto wheat spikelets at mid-anthesis. While the wild-type strains caused typical head blight symptoms 21 days after inoculation, five of the P450 deletion mutants (Δ*Fg03700*, Δ*Fg02111*, Δ*Fg00012*, Δ*Fg10451*, and Δ*Fg12737*) showed significantly reduced virulence compared with the wild type (Fig. 6).

All of the P450 deletion mutants were inoculated into rice media to analyze the production of secondary metabolites, such as deoxynivalenol (DON) and zearalenone. The levels of DON and zearalenone biosynthesized by P450 mutant strains were not significantly different compared with the wild-type strain according to conventional thin-layer chromatography (TLC) analysis (Supporting information Fig. S2), which was further confirmed by ELISA (Supporting information Table S2).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Fg02344
Fg11282
Fg07596
Fg08005
Fg08412
Fg11424
Fg03860
Fg01048
Fg00012
Fg02367
Fg10991
Fg07859
Fg02458
Fg05806
Fg03796
Fg11536
Fg09086
Fg12568
Fg09916
Fg11585
Fg03741
Fg06447
Fg03548
Fg01740
Fg09671
Fg01722
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Fg13445
Fg08186
Fg07977
Fg10571
Fg03260
Fg11303
Fg05960
Fg11108
Fg06068
CLM2
Fg04590
Fg11655
Fg01868
Fg01284
Fg01745
Fg01786
Fg10451
Fg04717
Fg08079
Fg01972
Fg02138
Fg00127
Fg02872
Fg08183
Fg11389
Fg08320
TRI4
Fg10461
Fg02419
Fg05113
Fg08023
TRI1
Fg02672
Fg03700
Fg12599
TRI11
Fg08187
Fg09033
Fg13739
Fg10910
Fg10629
Fg05334
Fg14006
Fg14007
Fg08207
CYP51C
Fg09195
Fg02929
Fg13176
Fg03191
Fg03542
Fg00863
Fg12371
Fg12372
Fg07765
Fg11002
Fg02982
Fg01767
Fg02117
Fg02111
Fg03086
Fg02118
Fg02114
Fg12534
Fg11038
Fg01739
CYP51B
Fg11465
Fg11498
Fg13796
Fg13797
Fg03498
Fg13459
Fg02668
Fg01583
Fg08377
Fg03686
Fg10695
Fg02113
Fg03264
CYP51A
Fg12283
Fg12284
Fg09351
Fg01959
Fg08191
Fg08809
Fg07804
Fg10960
A group
B group
2
1
0
-1
-2
6 h
9 h
12 h
1 d
2 d
3 d
4 d
5 d
Asexual development
Sexual development

FIG 3. Heatmap visualization of P450 gene transcriptional profiles during asexual and sexual development. The heatmap depicts P450 gene transcript levels during asexual and sexual developmental time points based on Log2-based relative transcript abundances compared with 3 h (asexual development) and 2 h (sexual development). Red represents higher expression; blue represents lower expression; and the rows represent transcriptional units. RNA-seq results were obtained from previous studies (Sikhakolli et al., 2012; Son et al., 2013; Son et al., 2016) and visualized using ClustVis (Metsalu and Vilo, 2015).

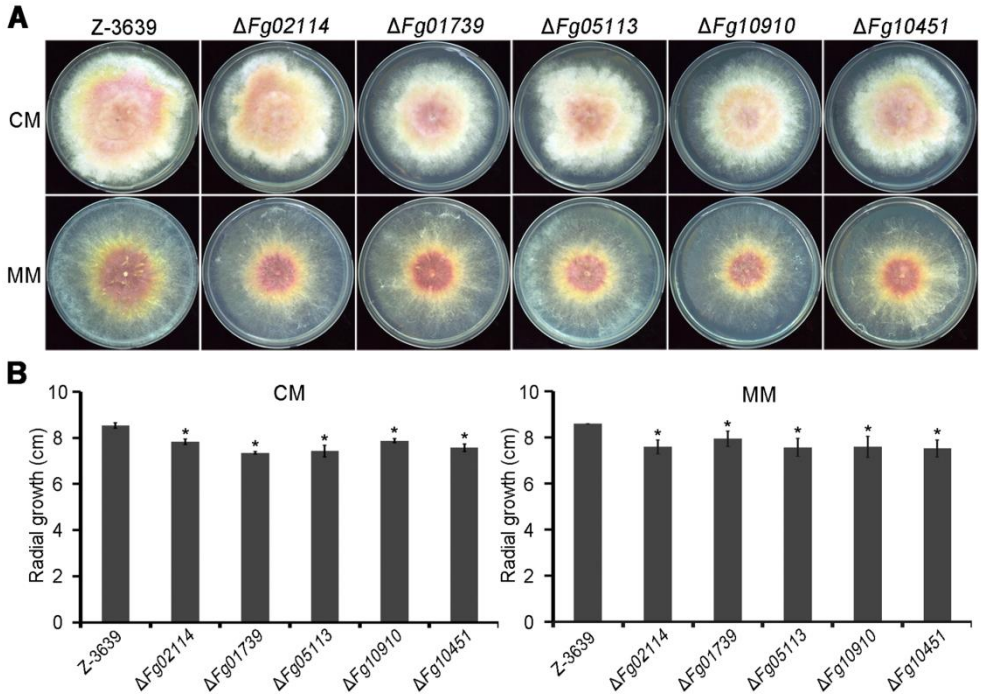


FIG 4. Vegetative growth of P450 mutants. A. Mycelial growth on complete medium (CM) and minimal medium (MM). Photographs were taken 5 days after inoculation. B. Radial growth on CM and MM. Radial growth was measured 5 days after inoculation. Significant differences ($p < 0.05$, *t* test) are indicated with an asterisk.

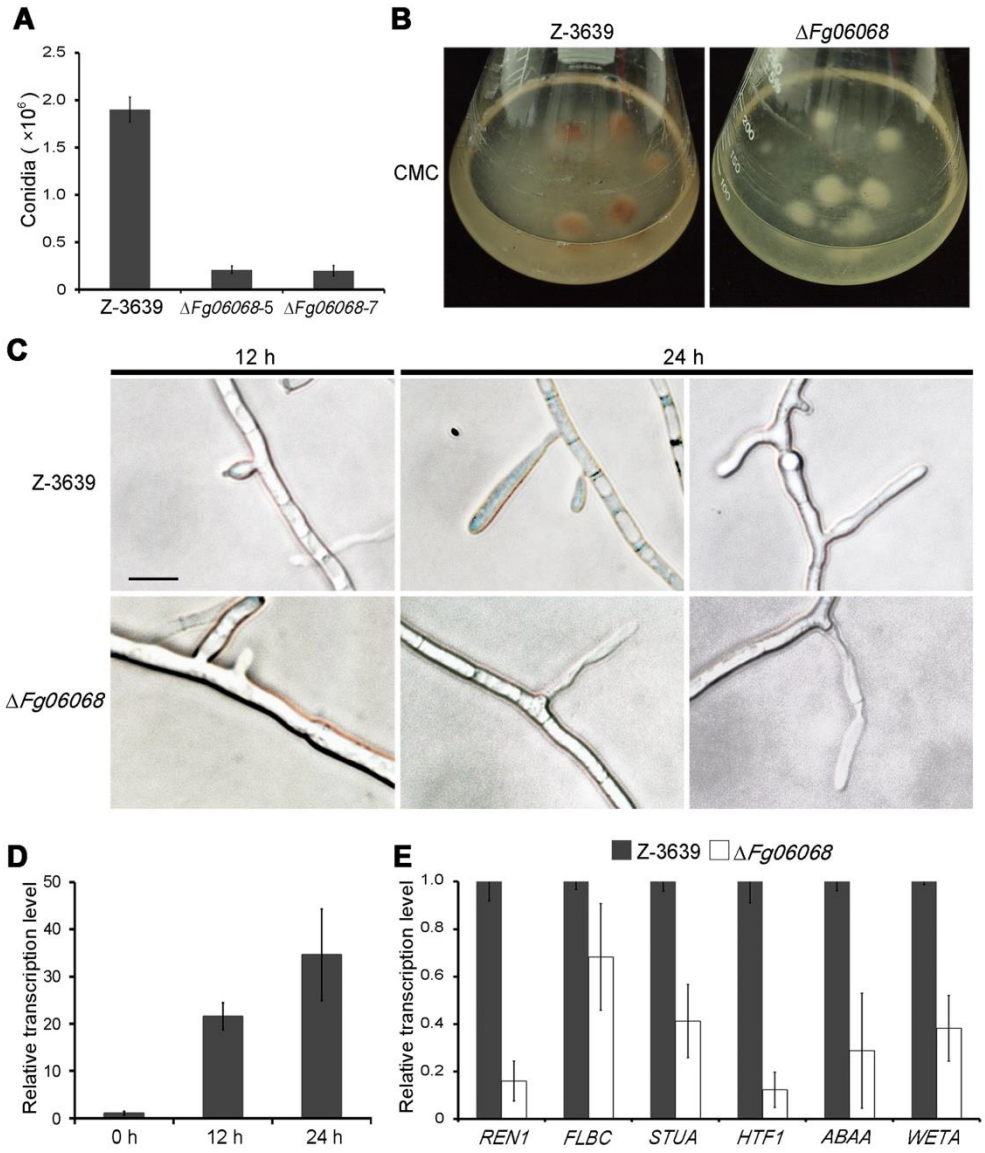

A
Conidia (×10^6)
2.5
2.0
1.5
1.0
0.5
0
Z-3639
ΔFg06068-5
ΔFg06068-7
B
Z-3639
ΔFg06068
CMC
C
12 h
24 h
Z-3639
ΔFg06068
D
Relative transcription level
50
40
30
20
10
0
0 h
12 h
24 h
E
Z-3639
ΔFg06068
Relative transcription level
1.0
0.8
0.6
0.4
0.2
0
REN1
FLBC
STUA
HTF1
ABAA
WETA

FIG 5. Defective conidiation of *Fg06068* deletion mutants. A. Conidium production. The number of conidia was counted at 5 days after inoculation in CMC. The values were generated based on three biological replicates. Significant differences ($p < 0.05$, *t* test) are indicated with an asterisk. B. Mycelial growth in CMC. The photographs were taken 3 days after inoculation. C. Morphology of conidiophores. The photographs were taken 1 to 2 days after conidium induction. Scale bar = 20 μm. D. Relative transcript levels of the *Fg06068* gene during asexual development. Total RNA was extracted from the wild type at 0, 6, 12, and 24 h after inoculation into CMC. E. Relative transcript levels of genes related to conidiation. Total RNA was extracted from the wild-type and Δ*Fg06068* strains at 18 h after inoculation into CMC. The relative transcript levels of each gene in the wild type were arbitrarily set to 1. Transcript levels were determined by qRT-PCR using two biological replicates with three technical repetitions each. Significant differences ($p < 0.05$, *t* test) are indicated with an asterisk.

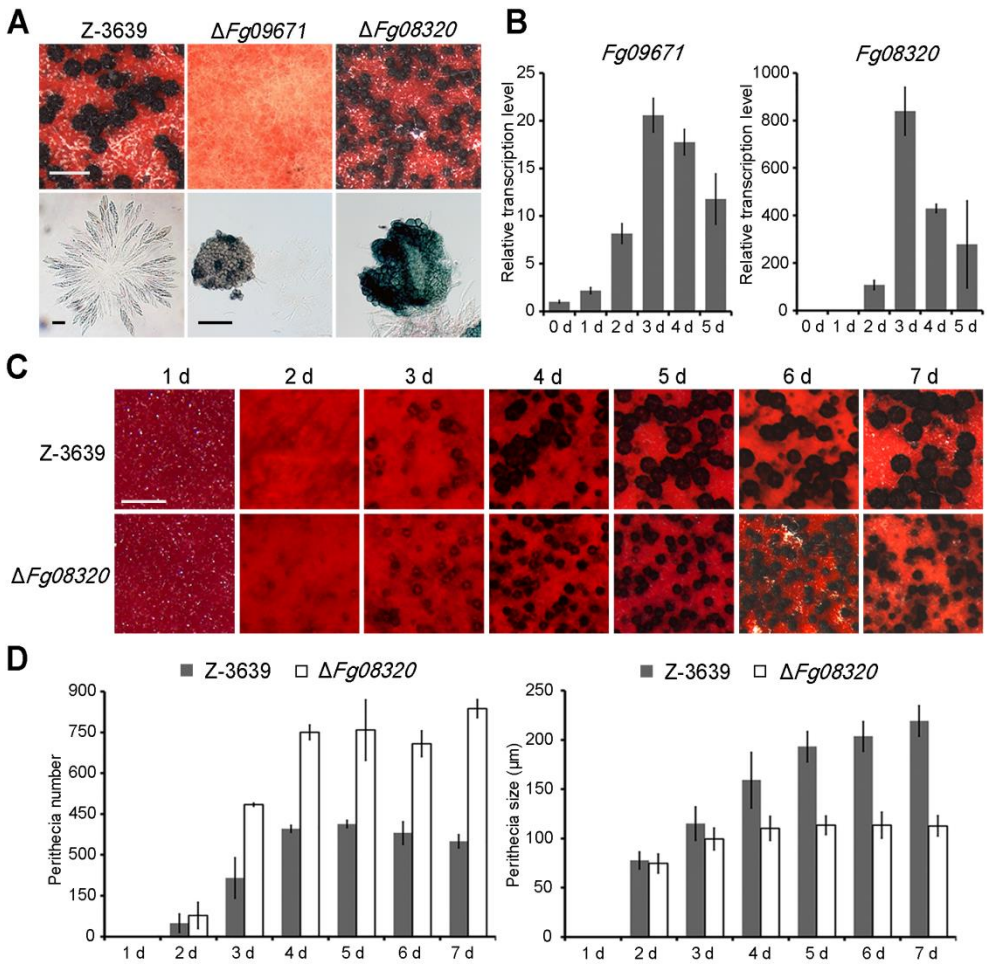
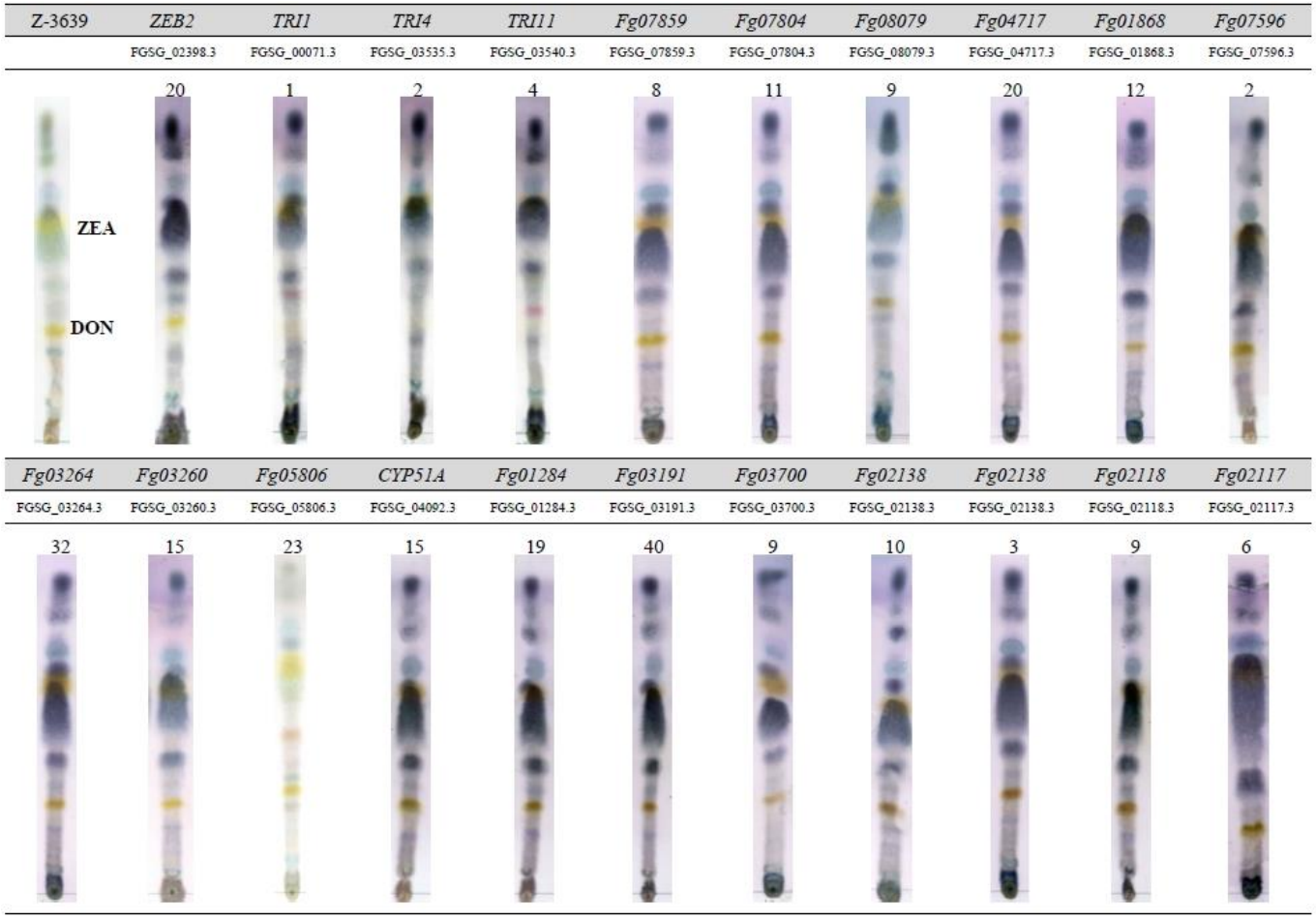


FIG 6. Sexual development of P450 mutants. A. Perithecium formation and asci rosettes of Z-3639, Δ*Fg09671*, and Δ*Fg08320*. The photographs were taken 7 days after sexual induction on carrot agar. Scale bar = 200 μm (upper panel) or 20 μm (lower panel). B. Transcript profiles of *Fg08320* and *Fg09671* during perithecium development. Total RNA was prepared 0~5 days after sexual induction, and the qRT-PCR was performed to analyze the transcript levels of each gene. C. Time-course of perithecium production. The photographs were taken every 24 h after

sexual induction. Scale bar = 200 μm. D. Time-course measurements of the number and size of perithecia. The diameters of the perithecia were measured for each strain using a dissecting microscope. The number of perithecia was counted from 30 mm^2 of carrot agar.

FIG 7. Virulence of *F. graminearum* strains in wheat heads. A center spikelet of each wheat head was injected with 10 μl of a conidial suspension. The photographs were taken 21 days after inoculation. The number of infected spikelets with significant differences ($p < 0.05$, *t* test) was determined 21 days after inoculation. Mock inoculation was performed with 0.01% Tween 20.


Z-3639
ZEB2
TRI1
TRI4
TRI11
Fg07859
Fg07804
Fg08079
Fg04717
Fg01868
Fg07596
FGSG_02398.3
FGSG_00071.3
FGSG_03535.3
FGSG_03540.3
FGSG_07859.3
FGSG_07804.3
FGSG_08079.3
FGSG_04717.3
FGSG_01868.3
FGSG_07596.3
20
1
2
4
8
11
9
20
12
2
ZEA
DON
Fg03264
Fg03260
Fg05806
CYP51A
Fg01284
Fg03191
Fg03700
Fg02138
Fg02138
Fg02118
Fg02117
FGSG_03264.3
FGSG_03260.3
FGSG_05806.3
FGSG_04092.3
FGSG_01284.3
FGSG_03191.3
FGSG_03700.3
FGSG_02138.3
FGSG_02138.3
FGSG_02118.3
FGSG_02117.3
32
15
23
15
19
40
9
10
3
9
6

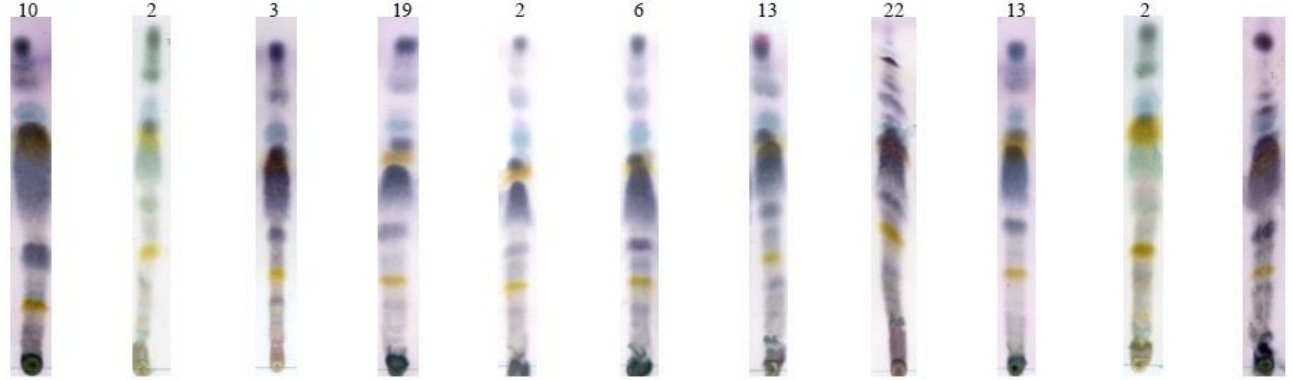
Fg05960	*Fg03860*	*Fg07765*	*Fg02982*	*Fg02114*	*Fg02111*	*Fg02458*	*Fg05334*	*Fg02672*	*Fg02668*	*Fg02929*
FGSG_05960.3	FGSG_03860.3	FGSG_07765.3	FGSG_02982.3	FGSG_02114.3	FGSG_02111.3	FGSG_02458.3	FGSG_05334.3	FGSG_02672.3	FGSG_02668.3	FGSG_02929.3
5	12	4	1	30	10	6	2	36	1	28
										

Fg06068	*Fg01786*	*Fg01767*	*Fg01745*	*Fg01740*	*Fg01739*	*Fg01722*	*Fg05113*	*Fg01583*	*Fg01048*	*Fg02419*
FGSG_06068.3	FGSG_01786.3	FGSG_01767.3	FGSG_01745.3	FGSG_01740.3	FGSG_01739.3	FGSG_01722.3	FGSG_05113.3	FGSG_01583.3	FGSG_01048.3	FGSG_02419.3
5	8	14	16	6	24	9	18	11	13	31
										

Fg02872	*Fg03548*	*Fg03542*	*Fg03489*	*Fg02371*	*Fg02367*	*Fg02366*	*Fg02344*	*Fg03796*	*Fg03741*	*Fg00863*
FGSG_02872.3	FGSG_03548.3	FGSG_03542.3	FGSG_03498.3	FGSG_02371.3	FGSG_02367.3	FGSG_02366.3	FGSG_02344.3	FGSG_03796.3	FGSG_03741.3	FGSG_00863.3
2	6	7	7	12	8	11	11	24	18	32
Fg02371	*Fg08320*	*Fg08207*	*Fg01972*	*Fg08187*	*CYP51B*	*Fg08186*	*Fg08183*	*CYP51C*	*Fg08005*	*Fg10629*
FGSG_08377.3	FGSG_08320.3	FGSG_08207.3	FGSG_01972.3	FGSG_08187.3	FGSG_01000.3	FGSG_08186.3	FGSG_08183.3	FGSG_07977.3	FGSG_08005.3	FGSG_10629.3
1	20	15	11	7	6	4	3	5	3	3

Fg00012	*CLM2*	*Fg00127*	*Fg08412*	*Fg08377*	*Fg10910*	*Fg11389*	*Fg11424*	*Fg11282*	*Fg11303*	*Fg11038*
FGSG_00012.3	FGSG_00007.3	FGSG_00127.3	FGSG_08412.3	FGSG_10695.3	FGSG_10910.3	FGSG_11389.3	FGSG_11424.3	FGSG_11282.3	FGSG_11303.3	FGSG_11038.3
14	15	16	51	4	21	1	17	9	2	3
Fg11024	*Fg11465*	*Fg11498*	*Fg11536*	*Fg10451*	*Fg10461*	*Fg09351*	*Fg09195*	*Fg09916*	*Fg09671*	*Fg11655*
FGSG_11024.3	FGSG_11465.3	FGSG_11498.3	FGSG_11536.3	FGSG_10451.3	FGSG_10461.3	FGSG_09351.3	FGSG_09195.3	FGSG_09916.3	FGSG_09671.3	FGSG_11655.3
13	37	6	12	32	1	5	6	24	3	5

Fg11108	*Fg10960*	*Fg10991*	*Fg11002*	*Fg12534*	*Fg12568*	*Fg08809*	*Fg13176*	*Fg12737*	*Fg12599*	*Fg13445*
FGSG_11108.3	FGSG_10960.3	FGSG_10991.3	FGSG_11002.3	FGSG_12534.3	FGSG_12568.3	FGSG_08809.3	FGSG_13176.3	FGSG_12737.3	FGSG_12599.3	FGSG_13445.3
10	2	3	19	2	6	13	22	13	2	



Fg13797	*Fg13459*	*Fg14007*	*Fg13739*	*Fg13796*
FGSG_13797.3	FGSG_13459.3	FGSG_14007.3	FGSG_13739.3	FGSG_13796.3
18	3	2	10	4

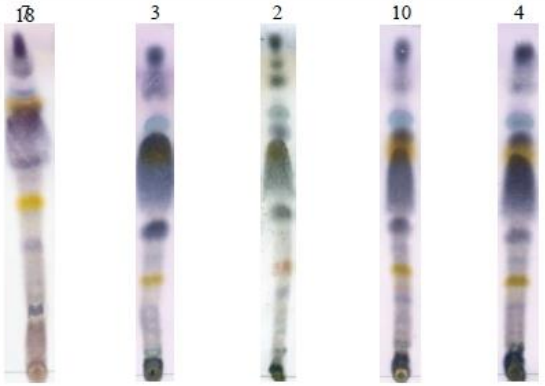


FIG 8. Mycotoxin analysis using thin-layer chromatography (TLC). Fungal strains were grown on a rice substrate for three weeks. Zearalenone (ZEA) and deoxynivalenol (DON) are indicated in a TLC plate image of the wild-type strain. The experiments were performed using two independent strains for each gene deletion with three biological replicates. *ZEB2* deleted mutant was used as a negative control for ZEA production. A representative TLC image of each gene deletion mutant is shown. WT, *F. graminearum* wild-type strain Z-3639. ZEA, zearalenone; DON, deoxynivalenol.

Table 2. Quantification of deoxynivalenol (DON) and zearalenone.

Gene name	Toxin production[a]	
	ZEA (µg/g)	**DON (µg/g)**
Z-3639[b]	1442.4 ± 159.1	477.1 ± 45.1
ZEB2[c]	0 ± 0	502.0 ± 13.8
TRI1	1455.7 ± 38.8	0 ± 0
TRI4	1260.5 ± 287.3	0 ± 0
TRI11	1299.7 ± 176.0	110.7 ± 17.8[d]
Fg02668	1396.2 ± 86.6	484.5 ± 10.7
Fg10960	1511.5 ± 21.1	475.6 ± 8.9
Z-3639	1486.1 ± 383.3	463.5 ± 69.2
Fg03264	1035.4 ± 197.9	408.3 ± 61.5
Fg03191	1464.0 ± 25.4	415.2 ± 11.2
Fg02118	1186.9 ± 57.4	461.2 ± 8.4
Fg11424	1545.9 ± 80.8	433.7 ± 9.0
Fg12568	1125.0 ± 140.6	434.9 ± 16.0
Fg07596	1753.1 ± 75.8	551.0 ± 31.6
CYP51A	1557.5 ± 44.9	450.7 ± 7.1
Fg01284	1534.7 ± 59.7	566.3 ± 11.4
Fg00127	1699.7 ± 28.9	397.1 ± 10.5
Fg10991	1703.6 ± 38.9	447.6 ± 4.5
Fg02982	1327.0 ± 180.7	450.5 ± 13.1
Fg03498	1188.0 ± 87.9	419.3 ± 4.7
Fg02419	1247.7 ± 144.8	484.0 ± 3.9
Fg10695	1220.4 ± 91.1	479.3 ± 31.5
Fg11465	1493.4 ± 48.3	487.6 ± 13.4
Fg11498	1657.2 ± 68.6	510.4 ± 15.7
Fg11536	1422.3 ± 332.3	493.3 ± 17.7
Fg10461	1708.6 ± 51.7	551.1 ± 34.9
Fg12534	1089.8 ± 431.4	490.1 ± 15.6
Fg00863	1127.6 ± 397.4	433.8 ± 36.1
Z-3639	1686.5 ± 66.0	599.2 ± 16.0
Fg02872	1681.8 ± 99.3	577.0 ± 23.2

Fg01767	1726.6 ± 65.2	584.9 ± 7.9
Fg01722	1708.2 ± 34.5	604.7 ± 14.6
Fg03686	1588.4 ± 45.5	598.6 ± 21.2
Fg02111	1568.6 ± 78.0	594.8 ± 17.7
Z-3639	1181.9 ± 158.0	390.1 ± 17.2
Fg11303	1143.7 ± 160.6	368.7 ± 11.3
Fg08005	1397.1 ± 57.7	372.8 ± 15.1
Z-3639	1537.1 ± 250.1	470.9 ± 32.5
Fg01048	1342.1 ± 190.4	432.1 ± 19.9
Fg08320	1356.5 ± 90.5	481.2 ± 1.8
Fg08186	1379.8 ± 110.7	465.6 ± 10.2
Fg05806	1364.8 ± 135.6	477.8 ± 7.4
Fg01786	1396.5 ± 170.6	461.9 ± 43.6
Fg11002	1327.1 ± 120.8	474.2 ± 6.4
Fg02138	1323.7 ± 67.5	498.6 ± 16.5
Fg13796	1341.5 ± 89.1	476.9 ± 5.0
Fg09671	1230.9 ± 199.0	460.1 ± 8.2
Fg09195	1349.8 ± 87.7	398.0 ± 125.0
Fg01972	1589.8 ± 170.0	480.4 ± 5.0
Fg08183	1470.8 ± 77.1	475.5 ± 10.5
CYP51B	1417.9 ± 132.8	477.8 ± 24.7
Fg04717	1498.7 ± 47.1	479.0 ± 7.3
Fg07804	1537.1 ± 106.1	473.7 ± 32.7
Fg03700	1536.2 ± 91.7	403.4 ± 85.4
Fg02366	1479.9 ± 69.3	453.2 ± 14.6
Fg01740	1558.8 ± 41.5	484.6 ± 6.6
Z-3639	1561.6 ± 79.3	533.5 ± 22.3
Fg02929	1688.7 ± 146	562.5 ± 18.2
Z-3639	1664.8 ± 149.9	495.1 ± 25.1
Fg03548	1683.9 ± 88.9	515.0 ± 18.4
Fg05334	1702.6 ± 105.1	486.4 ± 13.5
Fg02672	1667.1 ± 109.7	535.6 ± 14.8
Fg13739	1679.3 ± 111.7	435.2 ± 42.5
Fg05113	1627.0 ± 21.8	492.8 ± 10.3

Fg07859	1570.2 ± 201.3	512.1 ± 21.5
Fg05960	1509.7 ± 275.7	497.0 ± 69.2
Fg03860	1558.6 ± 25.2	511.8 ± 19.7
Z-3639	1360.9 ± 265.3	373.9 ± 43.6
Fg08079	1351.5 ± 51.8	408.2 ± 48.0
Fg02114	1024.8 ± 299.2	330.2 ± 6.4
Fg01745	1019.5 ± 176.7	316.3 ± 14.7
Fg01739	1697.1 ± 205.0	354.7 ± 29.9
Z-3639	1466.8 ± 112.0	476.5 ± 32.3
Fg09916	1126.9 ± 259.9	451.5 ± 45.0
Fg08187	1566.4 ± 36.4	480.4 ± 8.8
Fg02458	1590.9 ± 53.4	485.4 ± 2.1
Fg11024	1249.5 ± 150.3	484.2 ± 0.2
Fg02117	1640.6 ± 53.9	470.9 ± 18.9
Fg11282	1694.0 ± 58.6	481.8 ± 37.2
Fg10629	1385.7 ± 181.0	479.5 ± 7.5
Fg01583	1619.2 ± 81.4	489.4 ± 23.9
Fg13459	1461.9 ± 230.0	480.6 ± 16.5
CLM2	1637.5 ± 33.5	477.2 ± 14.2
Fg00012	1487.0 ± 16.4	489.1 ± 12.9
Fg02344	1487.9 ± 39.9	481.0 ± 7.9
Fg06068	1347.4 ± 108.5	465.5 ± 30.5
Fg03542	1304.9 ± 166.7	469.3 ± 17.5
Fg03260	1574.9 ± 49.6	499.8 ± 18.6
Fg12737	1716.5 ± 100.4	416.7 ± 52.8
Fg07765	1727.1 ± 69.9	497.1 ± 11.9
Fg11108	1708.5 ± 103.3	438.0 ± 74.7
Fg02367	1348.5 ± 253.0	404.2 ± 71.7
Z-3639	1602.6 ± 83.1	481 ± 38.3
Fg11655	1531.0 ± 159.6	471.1 ± 9.8
Fg13445	1613.0 ± 21.8	470.9 ± 2.6
Fg11038	1624.6 ± 65.9	486.1 ± 28.0
Fg12599	1622.1 ± 52.9	472.4 ± 9.1
Fg13176	1545.3 ± 53.9	481.0 ± 12.6

Fg08809	1612.5 ± 23.0	485.0 ± 13.3
Fg08377	1638.4 ± 117.1	502.5 ± 34.9
Fg11389	1678.4 ± 118.7	454.5 ± 19.6
Fg03741	1626.9 ± 147.0	486.7 ± 60.2
Fg09351	1585.2 ± 17.3	488.6 ± 27.3
Fg10451	1527.9 ± 55.7	461.4 ± 16.9
Fg13797	1449.5 ± 149.8	441.5 ± 19.7
Z-3639	1449.6 ± 202.2	468.3 ± 21.4
Fg02371	1280.9 ± 93.7	479.9 ± 37.0
Fg08412	1089.8 ± 164.9	420.4 ± 99.0
Fg08207	1769.8 ± 191.4	452.2 ± 17.8
Fg14007	1573.0 ± 93.3	458.2 ± 89.9
CYP51C	1522.3 ± 73.3	490.7 ± 17.7
Fg10910	1488.7 ± 128.1	466.0 ± 84.5
Fg01868	1640.7 ± 95.3	487.2 ± 61.2
Fg03796	1382.4 ± 79.7	489.8 ± 18.3

[a] Toxin production (ZEA, zearalenone; DON, deoxynivalenol) was quantified by Neogen Veratox kit and the experiments were performed using two independent strains with three biological repetitionsreplicates for each gene deletion.

[b] Mycotoxin production of each P450 mutant was not significantly different ($p < 0.05$, t test) compared to the wild type of corresponding experimental batch.

[c] ZEB2 deletion mutant is used for the negative control strains for ZEA production.

[d] Although ELISA signal for DON was detected in tri11 mutants possibly due to accumulated DON intermediates, further GC-MS analysis confirmed that tri11 deletion mutants did not produce DON.

V. Stress test

I performed in-depth phenotypic analyses under various environmental stresses. The tested conditions included osmotic (or ionic), reactive oxygen species (ROS), and cell wall stress; acidic (pH = 4) and basic (pH = 11) conditions; hot and cold conditions; and several fungicide treatments (Supporting information Table S1). None of the P450 mutants showed altered phenotypes under the evaluated stress conditions, with the exception of the Δ*Fg05960* mutant strain, which produced red pigments 1 day earlier than the wild type in the cold stress condition (data not shown).

As secondary metabolite biosynthetic P450 enzymes are related to UV stress responses in higher eukaryotes, such as humans and plants (Katiyar et al., 2000; Winkel-Shirley, 2001), I additionally tested P450 mutants under UV stress. The *Fg01583* gene deletion mutant grew faster than the wild-type strain under the UV-B stress condition and accumulated more orange pigment in its mycelia (Fig. 7A). Moreover, *Fg01583* was upregulated in response to UV light in the wild-type strain (Fig. 7B). The results suggest that the *Fg01583* gene plays a role in the UV stress response, and pigment accumulation is possibly involved in this process.

VI. Sensitivity to azole fungicides

Azole antifungal agents are well-known fungicides that inhibit fungal sterol 14α-demethylase (Cyp51) (Liu et al., 2011). To better understand the azole drug

resistance mechanism in *F. graminearum*, I screened P450 mutants on CM supplemented with five azole fungicides (tebuconazole, difenoconazole, epoxiconazole, propiconazole, and prochloraz). Experimental conditions were established in which the wild-type strain showed significant growth reduction compared with the control. In total, seventeen P450 mutants exhibited defective mycelial growth in at least one azole fungicide-complemented condition compared with the wild type (Supporting information Table S3). In particular, the Δ*Fg02114*, Δ*Fg02668*, Δ*Fg05113*, and Δ*Fg01048* strains displayed markedly increased sensitivity to several azole fungicides, including tebuconazole, difenoconazole, and prochloraz (Fig. 8). Three *CYP51* genes (*CYP51A*, *CYP51B*, and *CYP51C*) have been reported as *CYP51* paralogs, and only the Δ*cyp51A* strains showed increased sensitivity to azole drugs, as previously reported (Liu et al., 2011; Fan et al., 2013) (Fig. 8A and B).

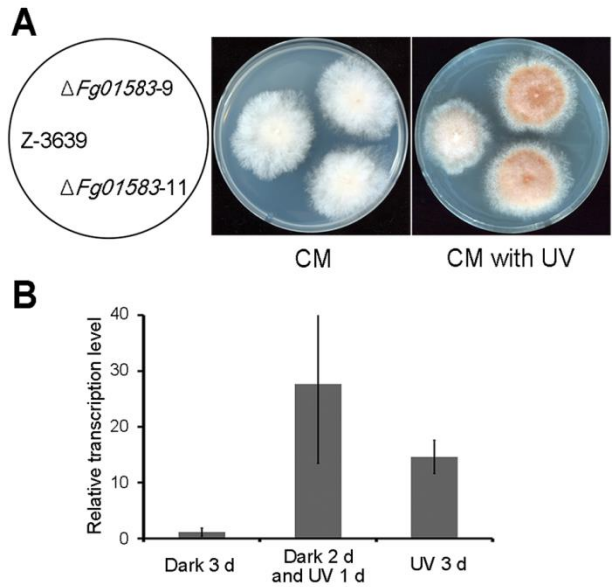


FIG 9. Altered UV stress response of *Fg01583* mutants. A. Mycelial growth in CM under UV light. The photographs were taken after 3 days of incubation under continuous UV-B light. B. Relative transcript levels of *Fg01583* in response to UV stress. Total RNA was extracted from the wild-type strain after 3 days of incubation in the dark (Dark 3 d); after 1 day of incubation under UV light after 2 days in the dark (Dark 2 d and UV 1 d); and after 3 days of incubation under UV light (UV 3 d) in CM.

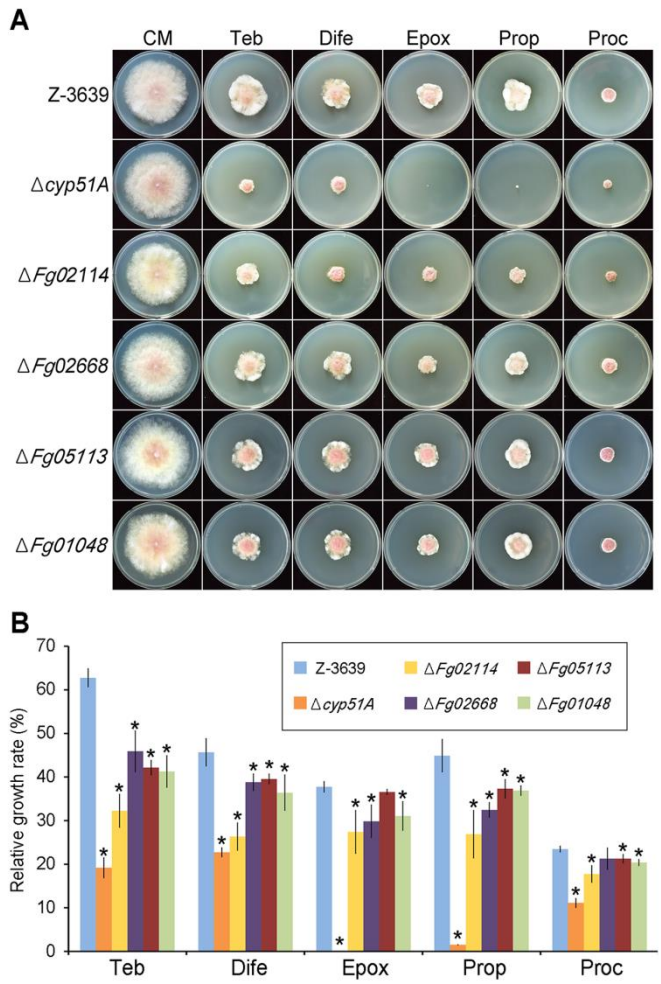


FIG 10. Azole fungicide sensitivity of *F. graminearum* strains. A. Mycelial growth of *F. graminearum* strains on CM with different azole fungicides. The photographs were taken 5 days after inoculation. B. Relative growth rate of *F. graminearum* strains on azole fungicide media compared with the control. Values with significant differences ($p < 0.05$, *t* test) compared with wild type are indicated by asterisks. Ten, tebuconazole; Dife, difenoconazole; Epox, epoxiconazole; Prop, propiconazole; Proc, prochloraz.

VII. Xenobiotic induction

Detoxification of xenobiotics (toxins, carcinogens, and pollutants) is one of the well-known functions of P450 enzymes (Werck-Reichhart and Feyereisen, 2000). To investigate the involvement of P450s in xenobiotic degradation, I screened my P450 mutant library with various xenobiotic compounds. I selected 15 xenobiotic compounds, including three aliphatics, four aromatics, five polyaromatics, two alkyl-substituted aromatics, and one alicyclic, for my analysis (Supporting information Table S4). Xenobiotic conditions were established in which the wild-type strain showed significant growth reduction compared with the control. Intriguingly, only one P450 gene deletion mutant was highly sensitive to CM containing 1-dodecanol compared with the wild type (Fig. 9A), and the *Fg01972* gene was markedly upregulated in response to 1-dodecanol in the wild-type strain (Fig. 9B).

Assuming that most P450s exhibit redundant functions in xenobiotic degradation, I compared the transcript abundance of P450 genes under 15 different xenobiotic stress conditions using quantitative real-time (qRT) PCR analysis (Supporting information Table S5 and Fig. 10). The expression levels of most P450 genes were significantly upregulated in at least one xenobiotic condition compared with the untreated control, and each xenobiotic condition induced many P450 genes at the same time (Fig. 10). While 1-dodecanol (DD), 4-octylphenol (OP), naphthalene (NPH) and 1-naphthol (NAP) induced most of the P450 genes, the majority of P450s were negatively regulated in response to treatments with

phenoxyacetic acid (PAA), resorcinol (RES), 3-methyl cholanthrene (MA), benzo(a)pyrene (BAP), sodium dodecylbenzenesulfonate (DBS), and n-dodecane (D). P450s included in the CYP505 (*Fg07596* and *Fg01972*) and CYP540 (*Fg02138* and *Fg03548*) families, which have been shown to be required for fatty acid hydroxylation (Nakayama et al., 1996), were highly induced in CM containing aliphatics (Supporting information Table S5 and Fig. 10).

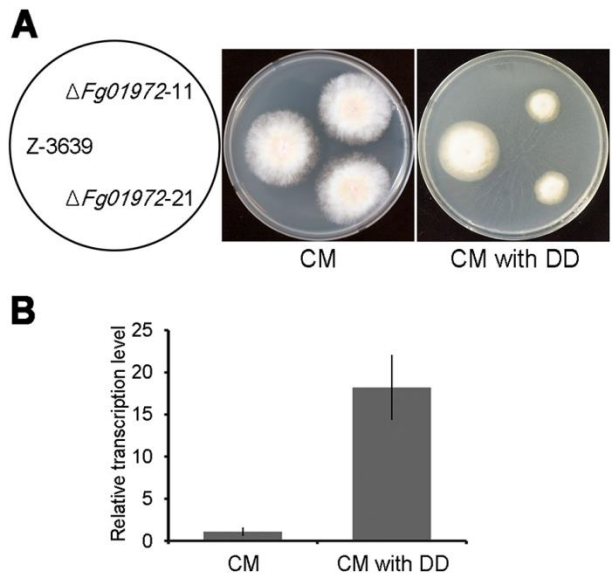
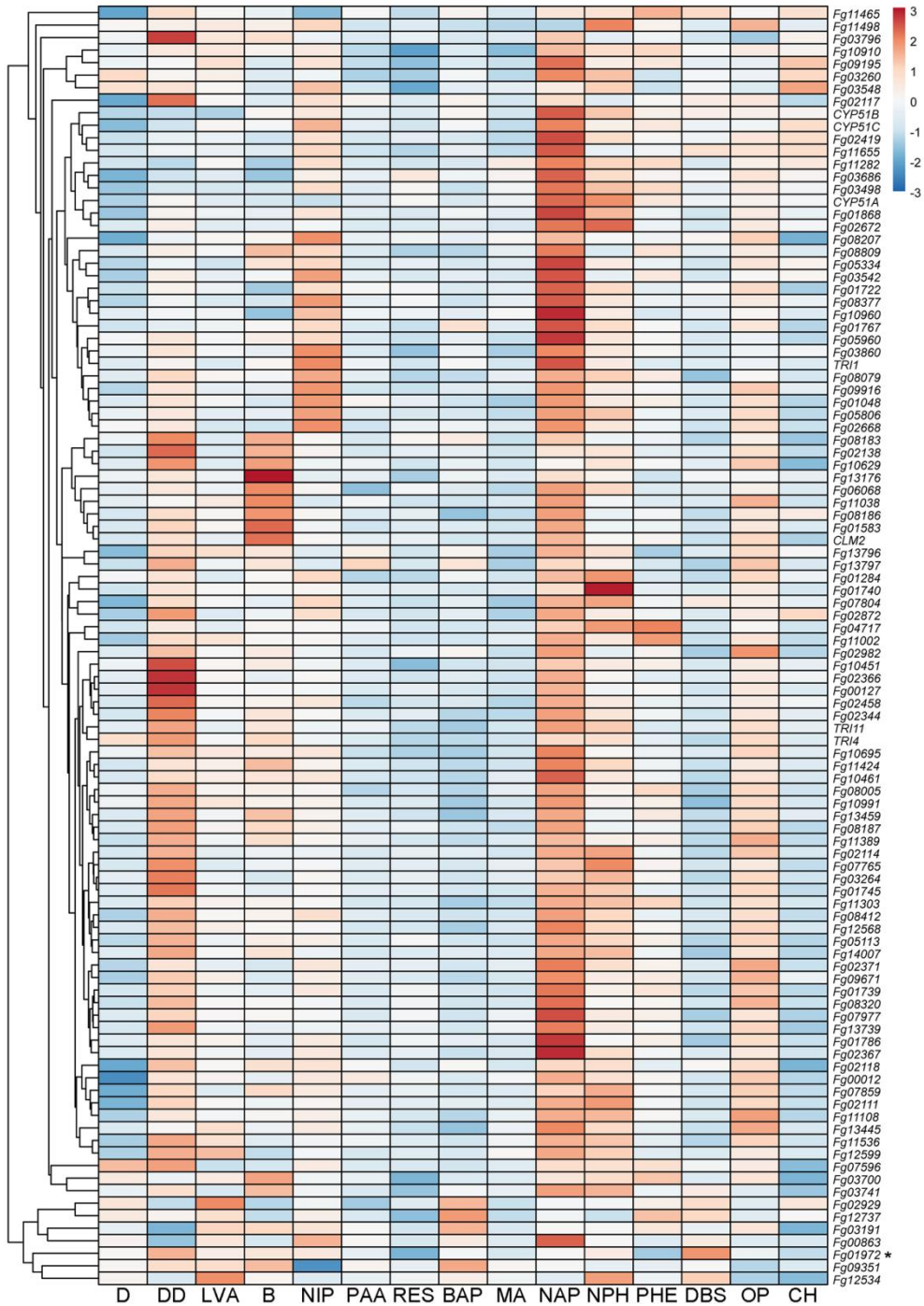


FIG 11. Altered xenobiotic stress response of the *Fg01972* mutants. A. Mycelial growth in Δ*Fg01972* on CM and CM supplemented with 1-dodecanol. The photographs were taken 3 days after inoculation. B. Relative transcript levels of the *Fg01972* gene in response to 1-dodecanol. Total RNA was extracted from the wild type 24 h after inoculation into CM with 1-dodecanol. DD, 1-dodecanol.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Fg10910
Fg09195
Fg03260
Fg03548
Fg02117
CYP51B
CYP51C
Fg02419
Fg11655
Fg11282
Fg03686
Fg03498
CYP51A
Fg01868
Fg02672
Fg08207
Fg08809
Fg05334
Fg03542
Fg01722
Fg08377
Fg10960
Fg01767
Fg05960
Fg03860
TRI1
Fg08079
Fg09916
Fg01048
Fg05806
Fg02668
Fg08183
Fg02138
Fg10629
Fg13176
Fg06068
Fg11038
Fg08186
Fg01583
CLM2
Fg13796
Fg13797
Fg01284
Fg01740
Fg07804
Fg02872
Fg04717
Fg11002
Fg02982
Fg10451
Fg02366
Fg00127
Fg02458
Fg02344
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Fg10695
Fg11424
Fg10461
Fg08005
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Fg07977
Fg13739
Fg01786
Fg02367
Fg02118
Fg00012
Fg07859
Fg02111
Fg11108
Fg13445
Fg11536
Fg12599
Fg07596
Fg03700
Fg03741
Fg02929
Fg12737
Fg03191
Fg00863
Fg01972 *
Fg09351
Fg12534
D
DD
LVA
B
NIP
PAA
RES
BAP
MA
NAP
NPH
PHE
DBS
OP
CH
3
2
1
0
-1
-2
-3

FIG 12. Heatmap visualization of P450 gene transcript levels under various xenobiotic conditions. The xenobiotic induction of P450 transcript levels was measured via qRT-PCR and visualized using ClustVis (Metsalu and Vilo, 2015) based on the Log2-based relative transcript abundance compared with the control. B, benzoate; DD, 1-dodecanol; OP, 4-octylphenol; NPH, naphthalene; PAA, phenoxy acetic acid; RES, resorcinol; MA, 3-methyl cholanthrene; BAP, benzo(a)pyrene; DBS, dodecyl benzene sulfonate; D, n-dodecane; LVA, levulinic acid; PHE, phenanthrene; CH, cyclohexanone; NIP, nitrophenol; NAP, 1-naphthol.

Table 3. Phenotypes of P450 mutants under various azole fungicide conditions.

Gene mame	Azole fungicides				
	Tebuconazole	**Difenoconazole**	**Epoxiconazole**	**Propiconazole**	**Procholoraz**
Fg08079	3[a]	4[b]	4[c]	4[d]	4[e]
Fg05960	4, cc[f]	4, cc	4, cc	4, cc	4, cc
CYP51A	1	2	0	1	2
Fg02114	2	2	3	2	3
Fg02672	4	4	4	4	3
Fg02668	3	3	3	3	4
Fg02929	3	4	4	3	3
Fg03498	3	4	4	4	4
Fg01767	3	4	4	4	4
Fg01745	3	4	3	4	4
Fg01740	3	4	4	3	4
Fg01739	3	4	4	4	4
Fg01722	3	4	4	4	4
Fg05113	3	3	4	3	4
Fg01583	3	4	4	4	4
Fg01048	3	3	3	3	4
Fg02419	3	4	4	4	4

[a] Percentage of average radial growth on CM with tebuconazole of deletion mutants compared to Z-3639 was scored (5, more than Z-3639; 4, 91-100%; 3, 61-90%; 2, 31-60 %; 1, 0-30 %; 0, 0 %).

[b] Percentage of average radial growth on CM with difenoconazole of deletion mutants compared to Z-3639 was scored (5, more than Z-3639; 4, 91-100%; 3, 61-90%; 2, 31-60 %; 1, 0-30 %; 0, 0 %).

[c] Percentage of average radial growth on CM with epoxiconazole of deletion mutants compared to Z-3639 was scored (5, more than Z-3639; 4, 91-100%; 3, 61-90%; 2, 31-60 %; 1, 0-30 %; 0, 0 %).

[d] Percentage of average radial growth on CM with propiconazole of deletion mutants compared toZ-3639 was scored (5, more than Z-3639; 4, 91-100%; 3, 61-90%; 2, 31-60 %; 1, 0-30 %; 0, 0 %).

[e] Percentage of average radial growth on CM with procholoraz of deletion mutants compared to Z-3639 was scored (5, more than Z-3639; 4, 91-100%; 3, 61-90%; 2, 31-60 %; 1, 0-30 %; 0, 0 %).

[f] Color change of pigment (cc)

Table 4. Transcription of P450 genes under xenobiotic induction.

Gene name	Locus number	Z-3639 DW	Z-3639 DMSO	Aliphatics			Aromatics						Polyaromatics				Alkyl-substituted aromatics		Alicyclics
				n-Dodecane	1-Dodecanol	Levulinic acid	Benzoate	Nitrophenol	Phenoxy acetic acid	Resorcinol	Benzo(a)pyrene	3-Methyl cholanthrene	1-Naphthol	Naphthalene	Phenanthrene	Dodecyl benzene sulfonate	Octylphenol	Cyclohexanone
Fg07859	FGSG_07859.3	1.11 ± 0.27	1.22 ± 0.37	0.26 ± 0.15	14.98 ± 0.95	1.69 ± 0.58	14.08 ± 5.32	7.09 ± 0.57	2.14 ± 0.15	2.25 ± 0.58	1.23 ± 0.23	1.51 ± 0.95	16.92 ± 0.58	31.95 ± 5.32	3.60 ± 0.95	1.22 ± 0.15	17.24 ± 0.23	0.79 ± 0.23
Fg07804	FGSG_07804.3	1.03 ± 0.28	1.28 ± 0.38	0.28 ± 0.36	4.16 ± 0.36	1.53 ± 1.40	1.13 ± 0.02	4.63 ± 2.85	0.88 ± 0.24	0.56 ± 0.40	1.06 ± 0.81	0.39 ± 0.17	7.58 ± 0.47	9.37 ± 3.84	1.42 ± 0.71	2.69 ± 0.79	2.41 ± 0.53	1.10 ± 0.17
Fg08079	FGSG_08079.3	1.28 ± 0.46	1.05 ± 0.25	1.3 ± 1.65	10.3 ± 2.99	3.2 ± 1.18	2.4 ± 0.10	23.5 ± 8.78	1.5 ± 0.53	0.5 ± 0.35	0.6 ±0.20	1.57 ± 0.48	21.40 ± 7.49	12.40 ± 5.45	4.56 ± 2.91	0.34 ± 0.10	2.88 ± 1.38	0.82 ± 0.41
Fg04717	FGSG_04717.3	1.07 ± 0.46	1.05 ± 0.34	1.42 ± 0.50	18.51 ± 5.04	3.23 ± 0.98	9.52 ± 3.01	8.94 ± 3.28	5.58 ± 1.98	1.41 ± 0.78	1.61 ± 1.16	2.04 ± 0.31	68.02 ± 6.06	230.74 ± 84.42	355.92 ± 245.75	1.47 ± 0.45	8.88 ± 2.81	1.46 ± 1.30
Fg01868	FGSG_01868.3	1.02 ± 0.20	1.24 ± 0.45	0.54 ± 0.06	2.28 ± 0.59	1.58 ± 0.49	1.38 ± 0.40	3.33 ± 1.04	1.16 ± 0.57	1.00 ± 0.62	1.71 ± 0.66	1.23 ± 0.52	18.23 ± 0.47	6.85 ± 2.12	1.60 ± 0.61	0.85 ± 0.28	2.68 ± 1.02	1.43 ± 0.55
Fg07596	FGSG_07596.3	1.19 ± 0.41	1.27 ± 0.65	122.44 ± 3.76	255.92 ± 170.78	0.76 ± 0.30	1.08 ± 0.81	15.45 ± 1.99	1.59 ± 0.54	1.48 ± 0.70	0.99 ± 0.11	2.10 ± 1.27	43.72 ± 27.53	34.86 ± 0.93	35.96 ± 24.19	5.20 ± 3.31	15.53 ± 3.63	0.20 ± 0.06
Fg05960	FGSG_05960.3	1.00 ± 0.06	1.03 ± 0.28	1.04 ± 0.12	1.76 ± 1.55	1.00 ± 0.02	1.46 ± 0.16	2.40 ± 0.08	0.61 ± 0.04	0.45 ± 0.18	0.78 ± 0.52	0.46 ± 0.16	9.94 ± 0.40	1.78 ± 0.57	1.14 ± 0.06	0.58 ± 0.07	0.89 ± 0.31	0.42 ± 0.44
Fg03860	FGSG_03860.3	1.00 ± 0.06	1.03 ± 0.25	0.87 ± 0.39	1.83 ± 1.75	1.03 ± 0.05	0.81 ± 0.63	4.94 ± 0.14	0.66 ± 0.09	0.34 ± 0.19	0.92 ± 0.34	0.39 ± 0.11	5.16 ± 0.20	1.66 ± 1.09	1.21 ± 0.13	0.64 ± 0.04	0.96 ± 0.32	1.05 ± 0.04
Fg07765	FGSG_07765.3	1.12 ± 0.15	1.10 ± 0.25	0.69 ± 0.48	115.15 ± 72.10	0.95 ± 0.29	1.80 ± 0.70	2.48 ± 0.85	0.99 ± 0.24	1.23 ± 0.58	0.65 ± 0.23	1.45 ± 0.47	29.96 ± 7.06	117.08 ± 20.44	4.17 ± 2.58	0.53 ± 0.32	6.97 ± 0.28	0.36 ± 0.12
Fg02982	FGSG_02982.3	1.02 ± 0.21	1.03 ± 0.26	0.86 ± 0.12	3.83 ± 0.65	1.36 ± 0.40	1.91 ± 0.68	1.21 ± 0.29	0.80 ± 0.23	1.16 ± 0.44	1.48 ± 1.26	0.62 ± 0.31	5.20 ± 1.26	1.01 ± 0.31	1.55 ± 0.42	0.50 ± 0.15	5.85 ± 2.05	0.53 ± 0.09
Fg03264	FGSG_03264.3	1.04 ± 0.28	1.10 ± 0.41	1.26 ± 0.74	40.16 ± 30.27	1.81 ± 0.47	0.88 ± 0.40	2.76 ± 0.46	1.04 ± 0.24	1.45 ± 1.06	0.70 ± 0.19	1.28 ± 0.39	11.81 ± 3.27	17.18 ± 15.29	2.83 ± 1.08	0.55 ± 0.35	9.38 ± 6.25	0.67 ± 0.32
Fg03260	FGSG_03260.3	1.04 ± 0.30	1.04 ± 0.30	5.47 ± 1.04	2.45 ± 0.18	2.87 ± 0.19	1.18 ± 1.03	1.85 ± 0.63	0.61 ± 0.14	0.57 ± 0.17	2.01 ± 0.79	0.67 ± 0.03	15.53 ± 4.75	7.68 ± 2.81	0.82 ± 0.25	2.05 ± 0.57	1.69 ± 1.09	5.70 ± 0.57
Fg05806	FGSG_05806.3	1.05 ± 0.35	1.13 ± 0.49	2.39 ± 0.35	7.38 ± 1.60	1.79 ± 0.43	2.04 ± 1.61	42.57 ± 0.99	1.44 ± 0.24	1.20 ± 0.72	1.21 ± 0.5	0.77 ± 0.11	42.57 ± 6.43	21.35 ± 11.68	2.81 ± 0.14	1.54 ± 0.47	13.91 ± 9.86	0.70 ± 0.10
CYP51A	FGSG_04092.3	2.07 ± 1.99	1.05 ± 0.39	0.32 ± 0.11	4.46 ± 3.69	1.29 ± 0.79	0.80 ± 0.61	0.48 ± 0.07	1.21 ± 0.31	3.91 ± 4.14	0.85 ± 0.88	2.07 ± 0.39	243.88 ± 30.13	127.33 ± 20.91	10.39 ± 4.26	2.54 ± 0.84	5.58 ± 2.16	3.21 ± 1.33
Fg01284	FGSG_01284.3	1.03 ± 0.26	1.03 ± 0.22	1.86 ± 0.56	3.23 ± 1.12	1.22 ± 0.05	1.97 ± 0.60	3.97 ± 1.35	0.81 ± 0.20	0.85 ± 0.34	1.77 ± 0.74	0.98 ± 0.37	5.15 ± 0.34	7.29 ± 2.57	1.14 ± 0.04	0.87 ± 0.59	3.98 ± 1.79	1.59 ± 0.53
Fg03191	FGSG_03191.3	1.06 ± 0.38	1.01 ± 0.16	0.73 ± 0.03	0.38 ± 0.27	1.44 ± 0.06	1.43 ± 0.11	1.23 ± 0.05	0.90 ± 0.35	0.57 ± 0.07	1.80 ± 1.34	0.62 ± 0.01	0.91 ± 0.33	0.91 ± 0.31	1.16 ± 0.04	0.76 ± 0.48	1.20 ± 0.05	0.38 ± 0.03
Fg03700	FGSG_03700.3	1.24 ± 0.61	1.13 ± 0.43	4.28 ± 1.69	1.55 ± 0.16	3.85 ± 0.11	21.48 ± 6.76	1.91 ± 0.70	1.44 ± 0.86	0.20 ± 0.14	0.87 ± 0.63	0.98 ± 0.38	5.16 ± 0.39	7.52 ± 2.45	12.03 ± 8.05	0.95 ± 0.34	5.22 ± 0.31	0.19 ± 0.11
Fg03686	FGSG_03686.3	1.25 ± 0.83	1.15 ± 0.63	0.23 ± 0.03	0.49 ± 0.42	0.74 ± 0.28	0.30 ± 0.20	1.67 ± 0.07	0.86 ± 0.52	2.18 ± 0.95	1.06 ± 0.74	1.50 ± 0.11	12.93 ± 0.15	3.11 ± 0.19	1.47 ± 0.04	1.19 ± 0.38	2.39 ± 0.82	1.49 ± 0.54

Fg02138	FGSG_02138.3	1.03 ± 0.26	1.05 ± 0.33	1.23 ± 0.10	119.59 ± 12.18	1.76 ± 0.53	38.06 ± 4.36	5.70 ± 0.55	1.48 ± 0.27	2.61 ± 2.23	2.79 ± 2.53	1.25 ± 0.03	11.75 ± 2.08	11.24 ± 1.31	2.37 ± 0.20	1.89 ± 0.57	15.30 ± 5.66	0.92 ± 0.27
Fg02118	FGSG_02118.3	1.01 ± 0.15	1.05 ± 0.32	0.47 ± 0.17	2.98 ± 0.59	1.44 ± 0.46	2.17 ± 1.99	2.10 ± 0.28	1.41 ± 0.31	0.82 ± 0.44	1.45 ± 0.94	0.90 ± 0.23	2.53 ± 0.14	2.28 ± 0.54	1.09 ± 0.11	1.45 ± 0.48	2.31 ± 0.34	0.51 ± 0.08
Fg02117	FGSG_02117.3	1.24 ± 0.60	1.02 ± 0.23	0.26 ± 0.07	10.23 ± 7.85	1.50 ± 0.54	0.83 ± 0.62	2.85 ± 0.43	1.67 ± 0.15	0.82 ± 0.63	1.78 ± 1.70	0.66 ± 0.21	3.13 ± 3.37	0.86 ± 0.03	1.50 ± 0.20	2.06 ± 1.29	2.36 ± 1.06	0.52 ± 0.03
Fg02114	FGSG_02114.3	1.01 ± 0.17	1.05 ± 0.36	0.69 ± 0.51	15.87 ± 3.47	1.09 ± 0.10	1.43 ± 0.63	1.38 ± 0.57	0.76 ± 0.21	1.15 ± 0.73	1.04 ± 1.26	0.62 ± 0.43	17.13 ± 3.78	20.60 ± 18.56	1.70 ± 0.73	0.40 ± 0.13	11.67 ± 8.43	0.66 ± 0.14
Fg02111	FGSG_02111.3	1.04 ± 0.31	1.04 ± 0.29	0.48 ± 0.13	6.69 ± 2.15	2.56 ± 0.48	2.01 ± 0.28	2.72 ± 0.28	1.67 ± 0.59	1.55 ± 0.99	1.57 ± 1.00	1.44 ± 0.14	10.22 ± 3.46	12.86 ± 8.44	2.29 ± 0.68	1.01 ± 0.26	6.24 ± 1.01	0.91 ± 0.30
Fg02458	FGSG_02458.3	1.04 ± 0.31	1.04 ± 0.33	1.24 ± 0.51	14.36 ± 6.00	2.48 ± 0.59	2.74 ± 0.61	3.71 ± 0.21	0.95 ± 0.20	1.14 ± 0.75	1.28 ± 0.88	0.89 ± 0.03	8.05 ± 0.59	3.38 ± 0.63	1.78 ± 0.26	1.49 ± 0.42	3.29 ± 0.20	2.05 ± 0.64
Fg05334	FGSG_05334.3	1.28 ± 0.68	1.03 ± 0.24	0.40 ± 0.21	0.96 ± 0.09	0.50 ± 0.04	2.13 ± 1.70	2.78 ± 0.10	0.65 ± 0.06	0.84 ± 0.58	0.57 ± 0.28	0.70 ± 0.06	10.81 ± 0.77	1.65 ± 1.36	0.94 ± 0.35	0.52 ± 0.13	2.00 ± 0.76	1.38 ± 0.41
Fg02672	FGSG_02672.3	1.03 ± 0.26	1.07 ± 0.34	1.40 ± 0.61	18.56 ± 2.75	2.04 ± 0.84	2.24 ± 0.84	6.50 ± 2.98	1.56 ± 0.46	1.46 ± 0.93	1.90 ± 1.37	1.38 ± 0.40	13699.26 ± 5314.40	1757.45 ± 720.12	4.12 ± 0.70	1.17 ± 0.71	22.50 ± 15.26	6.41 ± 4.26
Fg02668	FGSG_02668.3	1.08 ± 0.37	1.04 ± 0.29	1.43 ± 0.31	2.52 ± 0.36	0.70 ± 0.16	0.59 ± 0.19	9.19 ± 0.26	0.59 ± 0.05	1.10 ± 0.19	1.21 ± 0.74	0.89 ± 0.53	8.97 ± 0.66	2.63 ± 2.13	1.24 ± 0.13	0.57 ± 0.13	3.58 ± 2.02	0.54 ± 0.16
Fg02929	FGSG_02929.3	1.00 ± 0.02	1.16 ± 0.63	1.48 ± 0.58	0.43 ± 0.29	3.86 ± 0.06	0.41 ± 0.16	0.88 ± 0.03	0.36 ± 0.17	0.64 ± 0.18	2.58 ± 0.96	0.46 ± 0.05	0.91 ± 0.04	0.90 ± 0.04	1.15 ± 0.38	1.74 ± 0.18	0.65 ± 0.22	1.44 ± 0.50
Fg02872	FGSG_02872.3	1.06 ± 0.38	1.02 ± 0.24	0.48 ± 0.16	8.86 ± 1.54	0.94 ± 0.31	1.34 ± 1.10	3.47 ± 0.18	0.82 ± 0.12	0.69 ± 0.36	1.07 ± 0.63	0.49 ± 0.04	7.50 ± 0.62	2.39 ± 1.58	1.43 ± 0.59	0.89 ± 0.10	2.43 ± 0.88	4.34 ± 1.59
Fg03548	FGSG_03548.3	1.01 ± 0.11	1.05 ± 0.33	14.04 ± 1.35	12.46 ± 3.23	5.49 ± 1.93	2.01 ± 0.20	26.83 ± 17.23	1.70 ± 1.11	0.42 ± 0.28	2.70 ± 0.13	2.90 ± 2.45	8.28 ± 2.85	21.72 ± 0.89	1.27 ± 0.20	4.23 ± 1.55	2.07 ± 0.89	42.59 ± 14.62
Fg03542	FGSG_03542.3	1.06 ± 0.37	1.00 ± 0.10	0.26 ± 0.11	2.87 ± 1.97	1.08 ± 0.42	1.61 ± 1.47	21.76 ± 9.16	0.97 ± 0.07	0.60 ± 0.41	0.91 ± 0.79	0.60 ± 0.19	63.25 ± 3.01	1.03 ± 0.55	3.93 ± 0.30	0.50 ± 0.04	1.88 ± 0.20	2.18 ± 0.74
TRI11	FGSG_03540.3	1.06 ± 0.39	1.01 ± 0.13	1.89 ± 0.44	22.36 ± 13.00	2.11 ± 0.74	6.442 ± 2.49	2.57 ± 0.80	2.49 ± 1.26	0.84 ± 0.15	0.66 ± 0.23	1.20 ± 0.40	24.80 ± 8.98	12.86 ± 9.39	1.53 ± 0.54	1.64 ± 0.34	8.95 ± 5.22	1.94 ± 0.65
TRI4	FGSG_03535.3	1.05 ± 0.35	1.02 ± 0.23	8.68 ± 3.80	37.62 ± 23.15	2.24 ± 0.78	11.84 ± 6.27	1.90 ± 0.55	0.91 ± 0.51	0.41 ± 0.02	0.47 ± 0.09	0.63 ± 0.26	10.17 ± 3.12	8.76 ± 6.54	1.40 ± 0.45	0.46 ± 0.25	10.19 ± 7.90	0.93 ± 0.31
Fg03498	FGSG_03498.3	1.26 ± 0.85	1.15 ± 0.62	0.29 ± 0.19	0.85 ± 0.87	0.90 ± 0.10	0.92 ± 0.96	14.99 ± 10.45	1.22 ± 0.86	3.91 ± 2.31	0.56 ± 0.39	2.63 ± 0.91	117.57 ± 3.59	29.92 ± 0.59	16.53 ± 9.28	1.86 ± 0.17	6.97 ± 0.66	2.79 ± 0.99
Fg06068	FGSG_06068.3	1.07 ± 0.32	1.14 ± 0.43	1.22 ± 0.45	3.14 ± 2.95	1.31 ± 0.52	13.96 ± 0.25	2.01 ± 0.67	0.33 ± 0.02	1.59 ± 1.13	0.97 ± 0.43	0.52 ± 0.17	10.75 ± 0.78	5.12 ± 0.22	0.95 ± 0.34	0.97 ± 0.26	2.46 ± 0.36	0.84 ± 0.05
Fg01786	FGSG_01786.3	1.06 ± 0.31	1.18 ± 0.51	0.90 ± 0.46	2.31 ± 0.34	1.65 ± 0.44	0.96 ± 0.85	1.98 ± 0.88	0.75 ± 0.32	1.28 ± 0.86	1.05 ± 0.72	0.77 ± 0.33	11.87 ± 1.25	1.41 ± 0.55	1.77 ± 0.58	0.46 ± 0.28	2.88 ± 0.16	0.63 ± 0.45
Fg01767	FGSG_01767.3	1.04 ± 0.32	1.47 ± 0.78	0.49 ± 0.29	0.78 ± 0.69	0.97 ± 0.29	1.23 ± 0.88	2.78 ± 0.30	0.63 ± 0.06	0.47 ± 0.20	2.39 ± 0.32	0.71 ± 0.42	10.46 ± 0.78	2.62 ± 0.11	1.19 ± 0.08	0.56 ± 0.12	1.95 ± 0.73	0.40 ± 0.03
Fg01745	FGSG_01745.3	1.04 ± 0.32	1.37 ± 0.76	1.64 ± 1.10	73.25 ± 58.11	3.14 ± 0.34	2.03 ± 0.76	3.99 ± 0.99	1.80 ± 0.47	1.97 ± 0.71	1.13 ± 0.57	1.18 ± 0.32	27.76 ± 8.61	18.33 ± 13.94	2.49 ± 0.15	1.05 ± 0.34	14.28 ± 9.88	0.82 ± 0.13
Fg01740	FGSG_01740.3	1.03 ± 0.25	1.23 ± 1.05	0.68 ± 0.35	14.13 ± 7.94	4.20 ± 1.14	4.59 ± 3.79	5.20 ± 3.22	1.65 ± 1.15	1.45 ± 1.05	0.93 ± 0.42	1.25 ± 0.37	18.95 ± 4.61	1059 ± 5.55	2.13 ± 0.89	1.18 ± 0.90	9.46 ± 6.72	0.82 ± 0.26

Fg01739	FGSG_01739.3	1.01 ± 0.11	1.20 ± 1.22	1.12 ± 0.89	6.93 ± 2.23	2.07 ± 0.31	1.58 ± 0.40	3.95 ± 2.16	1.29 ± 0.36	2.18 ± 1.43	1.18 ± 1.02	1.06 ± 0.21	17.69 ± 1.57	3.18 ± 0.95	4.46 ± 1.02	0.82 ± 0.28	7.72 ± 0.30	0.84 ± 0.12
Fg01722	FGSG_01722.3	1.05 ± 0.38	1.31 ± 0.79	1.07 ± 0.92	4.52 ± 0.03	2.64 ± 0.56	0.72 ± 0.13	9.40 ± 2.70	3.26 ± 1.88	3.09 ± 2.22	1.63 ± 0.42	1.98 ± 0.82	47.59 ± 6.62	10.23 ± 0.57	2.12 ± 0.42	3.44 ± 0.69	9.76 ± 0.64	0.78 ± 0.27
Fg05113	FGSG_05113.3	1.13 ± 0.49	1.11 ± 0.66	0.77 ± 0.41	16.65 ± 9.01	2.14 ± 1.58	4.35 ± 2.09	4.11 ± 1.55	1.30 ± 0.46	2.93 ± 2.67	1.11 ± 0.62	0.95 ± 0.12	18.35 ± 8.01	9.61 ± 6.07	4.11 ± 2.26	0.70 ± 0.31	9.67 ± 5.42	0.73 ± 0.25
Fg01583	FGSG_01583.3	1.03 ± 0.27	1.05 ± 0.64	0.90 ± 0.58	6.90 ± 1.63	2.36 ± 0.61	27.55 ± 2.40	1.78 ± 0.41	0.68 ± 0.28	1.03 ± 0.29	1.05 ± 0.28	0.73 ± 0.30	12.72 ± 3.76	1.64 ± 0.46	1.66 ± 0.29	0.72 ± 0.33	3.78 ± 0.99	0.96 ± 0.20
Fg01048	FGSG_01048.3	1.00 ± 0.03	1.16 ± 0.53	0.76 ± 0.56	1.46 ± 1.36	1.01 ± 0.06	0.71 ± 0.69	2.63 ± 0.75	1.11 ± 0.21	0.85 ± 0.39	0.64 ± 0.23	0.53 ± 0.12	2.64 ± 0.77	1.35 ± 0.97	1.23 ± 0.33	0.68 ± 0.19	1.62 ± 0.60	0.55 ± 0.02
Fg02419	FGSG_02419.3	1.03 ± 0.30	1.30 ± 0.78	0.82 ± 0.06	2.41 ± 0.66	2.55 ± 0.88	0.92 ± 0.79	10.74 ± 0.44	1.23 ± 0.18	1.90 ± 0.79	1.26 ± 0.24	0.73 ± 0.05	133.62 ± 47.33	13.82 ± 8.73	4.03 ± 1.47	2.94 ± 0.27	8.40 ± 3.22	13.21 ± 0.69
Fg02371	FGSG_02371.3	1.17 ± 0.51	1.21 ± 0.84	1.50 ± 1.17	15.96 ± 5.67	4.33 ± 0.65	2.41 ± 1.94	14.26 ± 3.93	3.83 ± 0.43	4.40 ± 3.13	2.33 ± 1.94	3.81 ± 1.33	97.42 ± 16.35	18.68 ± 6.77	8.79 ± 4.19	2.82 ± 3.09	53.47 ± 21.35	1.79 ± 0.73
Fg02367	FGSG_02367.3	1.04 ± 0.34	1.08 ± 0.52	1.28 ± 0.98	12.86 ± 2.06	3.29 ± 2.35	1.78 ± 1.04	10.65 ± 4.76	1.36 ± 0.21	2.00 ± 1.26	0.67 ± 0.37	1.08 ± 0.32	667.88 ± 14.56	19.48 ± 1.98	3.66 ± 1.19	1.28 ± 0.67	11.17 ± 1.14	0.78 ± 0.51
Fg02366	FGSG_02366.3	1.14 ± 0.64	1.16 ± 0.62	1.06 ± 0.56	35.77 ± 28.45	2.09 ± 0.82	1.19 ± 0.17	1.97 ± 0.48	0.95 ± 0.32	0.65 ± 0.42	1.01 ± 0.21	0.62 ± 0.15	9.62 ± 3.54	2.04 ± 0.51	1.10 ± 0.33	0.95 ± 0.11	2.81 ± 0.53	1.59 ± 0.49
Fg02344	FGSG_02344.3	1.05 ± 0.35	1.15 ± 0.66	1.16 ± 0.72	35.75 ± 27.52	2.91 ± 0.49	6.76 ± 5.21	3.44 ± 1.57	1.38 ± 0.46	1.77 ± 1.26	0.75 ± 0.42	0.82 ± 0.10	25.61 ± 2.59	6.74 ± 0.42	2.74 ± 0.60	1.01 ± 0.33	6.39 ± 1.23	1.76 ± 0.30
Fg03796	FGSG_03796.3	1.02 ± 0.20	1.21 ± 0.46	1.10 ± 0.87	39.58 ± 31.11	2.71 ± 0.72	3.44 ± 2.57	0.91 ± 0.35	0.61 ± 0.12	0.90 ± 0.65	0.50 ± 0.18	0.58 ± 0.06	5.99 ± 0.62	0.68 ± 0.05	1.09 ± 0.53	0.42 ± 0.18	0.21 ± 0.34	1.73 ± 0.15
Fg03741	FGSG_03741.3	1.00 ± 0.04	1.04 ± 0.29	1.12 ± 0.11	2.84 ± 1.79	0.80 ± 0.29	4.52 ± 2.03	1.32 ± 0.05	0.79 ± 0.50	0.40 ± 0.16	1.34 ± 0.02	1.05 ± 0.44	6.03 ± 0.53	4.98 ± 0.28	1.25 ± 0.08	1.99 ± 0.63	0.93 ± 0.38	0.43 ± 0.17
Fg00863	FGSG_00863.3	1.06 ± 0.39	1.19 ± 0.52	1.07 ± 0.37	0.33 ± 0.27	1.46 ± 0.03	0.56 ± 0.21	2.68 ± 0.11	0.94 ± 0.31	0.55 ± 0.11	1.34 ± 0.12	0.64 ± 0.02	4.91 ± 0.17	0.95 ± 0.36	0.63 ± 0.02	1.31 ± 0.09	0.64 ± 0.01	0.57 ± 0.21
Fg00012	FGSG_00012.3	1.14 ± 0.57	1.03 ± 0.72	0.18 ± 0.09	3.38 ± 2.92	2.07 ± 0.44	1.21 ± 0.93	2.88 ± 0.85	2.76 ± 1.25	1.49 ± 1.35	0.90 ± 0.10	1.60 ± 0.34	7.67 ± 0.79	3.51 ± 2.08	1.88 ± 0.46	0.75 ± 0.20	5.33 ± 2.27	0.52 ± 0.30
CLM2	FGSG_00007.3	1.02 ± 0.23	1.29 ± 0.53	1.30 ± 0.98	8.57 ± 0.90	2.23 ± 0.55	45.72 ± 18.56	3.58 ± 1.46	2.03 ± 0.43	2.36 ± 2.24	1.21 ± 0.60	1.31 ± 0.63	18.49 ± 7.41	3.64 ± 0.53	3.96 ± 1.64	1.00 ± 0.72	11.23 ± 2.04	0.75 ± 0.30
TRI1	FGSG_00072.3	1.00 ± 0.04	1.00 ± 0.07	1.05 ± 0.10	2.22 ± 1.46	0.96 ± 0.04	2.14 ± 1.26	15.07 ± 1.69	1.52 ± 0.51	0.73 ± 0.29	1.93 ± 0.06	0.80 ± 0.32	24.32 ± 9.92	2.90 ± 1.02	1.05 ± 0.10	1.05 ± 0.06	1.52 ± 0.59	0.95 ± 0.06
Fg00127	FGSG_00127.3	1.04 ± 0.30	1.07 ± 0.38	0.89 ± 0.54	49.42 ± 38.87	1.61 ± 0.29	1.59 ± 1.51	1.45 ± 0.43	0.70 ± 0.31	1.00 ± 0.83	0.60 ± 0.31	0.54 ± 0.25	9.77 ± 3.94	1.38 ± 0.24	1.66 ± 1.11	0.54 ± 0.20	3.18 ± 0.17	0.72 ± 0.50
Fg08412	FGSG_08412.3	1.00 ± 0.07	1.48 ± 0.83	0.76 ± 0.34	30.75 ± 19.51	4.41 ± 2.19	5.27 ± 3.32	11.51 ± 1.63	1.86 ± 1.02	1.55 ± 1.23	1.11 ± 0.46	1.31 ± 0.40	40.75 ± 1.07	14.85 ± 10.71	2.73 ± 1.58	1.55 ± 0.46	12.46 ± 8.28	0.97 ± 0.21
Fg08377	FGSG_08377.3	1.08 ± 0.45	1.03 ± 0.27	0.35 ± 0.03	1.28 ± 1.08	0.68 ± 0.02	0.57 ± 0.17	12.34 ± 8.03	0.93 ± 0.38	1.48 ± 1.34	0.47 ± 0.21	0.95 ± 0.35	25.49 ± 16.41	2.70 ± 0.20	1.18 ± 0.08	0.97 ± 0.33	2.57 ± 0.09	1.11 ± 0.44
Fg08320	FGSG_08320.3	1.03 ± 0.26	1.17 ± 0.64	0.76 ± 0.42	6.73 ± 1.85	1.96 ± 0.55	2.05 ± 0.88	1.86 ± 0.44	1.08 ± 0.25	1.71 ± 1.42	0.85 ± 0.46	0.94 ± 0.50	14.13 ± 1.02	1.98 ± 1.01	2.10 ± 0.92	0.90 ± 0.50	7.27 ± 1.09	0.78 ± 0.42
Fg08207	FGSG_08207.3	1.40 ± 0.58	1.82 ± 1.02	0.27 ± 0.17	1.39 ± 0.74	2.01 ± 0.61	2.00 ± 1.79	13.22 ± 0.55	1.33 ± 0.52	1.65 ± 1.44	1.21 ± 0.63	1.97 ± 1.19	7.38 ± 1.16	1.82 ± 0.12	1.56 ± 0.40	2.03 ± 0.80	5.80 ± 3.65	0.28 ± 0.20

Fg01972	FGSG_01972.3	1.07 ± 0.43	1.40 ± 1.07	1.91 ± 1.35	18.21 ± 3.79	2.98 ± 0.29	4.18 ± 1.77	4.08 ± 1.45	1.00 ± 0.40	0.11 ± 0.09	1.60 ± 1.22	0.49 ± 0.19	1.87 ± 0.72	3.24 ± 1.00	0.20 ± 0.06	31.64 ± 10.35	1.33 ± 0.05	0.32 ± 0.05
Fg08187	FGSG_08187.3	1.00 ± 0.05	1.18 ± 0.62	0.67 ± 0.47	6.34 ± 0.15	1.53 ± 0.37	3.19 ± 0.16	1.79 ± 0.15	0.85 ± 0.17	1.04 ± 0.85	0.56 ± 0.40	0.49 ± 0.07	6.33 ± 1.48	1.13 ± 0.24	1.19 ± 0.51	0.48 ± 0.09	3.78 ± 0.48	0.47 ± 0.15
CYP51B	FGSG_01000.3	1.07 ± 0.42	1.17 ± 0.66	0.27 ± 0.09	0.33 ± .28	0.26 ± 0.88	2.02 ± 0.63	3.70 ± 0.11	0.86 ± 0.08	0.60 ± 0.34	2.01 ± 1.74	0.44 ± 0.02	57.49 ± 2.24	10.65 ± 3.62	2.48 ± 1.84	2.70 ± 0.86	2.65 ± 0.96	2.08 ± 0.70
Fg08186	FGSG_08186.3	1.21 ± 0.52	1.12 ± 0.67	1.10 ± 0.69	6.21 ± 0.64	2.81 ± 0.81	16.46 ± 3.58	2.71 ± 0.79	1.25 ± 0.41	1.63 ± 1.25	0.53 ± 0.39	0.81 ± 0.09	15.11 ± 2.94	1.77 ± 0.24	2.33 ± 1.10	0.83 ± 0.69	4.71 ± 1.15	3.84 ± 3.13
Fg08183	FGSG_08183.3	1.08 ± 0.47	1.27 ± 0.62	0.71 ± 0.70	10.93 ± 4.90	0.43 ± 0.16	6.35 ± 0.61	0.95 ± 0.30	0.34 ± 0.14	1.07 ± 0.50	1.56 ± 1.38	0.25 ± 0.04	4.17 ± 0.89	0.74 ± 0.35	0.65 ± 0.22	0.19 ± 0.11	1.32 ± 0.23	0.14 ± 0.05
CYP51C	FGSG_07977.3	1.16 ± 0.70	1.03 ± 0.80	0.95 ± 0.74	5.91 ± 0.25	2.00 ± 0.94	2.00 ± 1.41	1.92 ± 0.12	0.86 ± 0.28	1.72 ± 1.61	0.68 ± 0.30	1.46 ± 0.63	29.70 ± 3.02	3.23 ± 0.39	2.70 ± 1.61	0.44 ± 0.27	3.88 ± 0.44	0.48 ± 0.23
Fg08005	FGSG_08005.3	1.32 ± 0.72	1.21 ± 0.36	1.81 ± 1.70	11.13 ± 3.78	2.86 ± 0.64	3.09 ± 1.10	2.62 ± 0.81	0.70 ± 0.54	1.03 ± 0.63	0.71 ± 0.60	1.59 ± 1.13	12.52 ± 0.63	3.05 ± 0.48	5.96 ± 1.91	0.60 ± 0.56	6.54 ± 1.21	0.90 ± 0.47
Fg10629	FGSG_10629.3	1.13 ± 0.64	1.26 ± 0.87	1.06 ± 1.31	37.03 ± 7.59	1.07 ± 0.28	31.02 ± 21.62	1.82 ± 1.86	1.62 ± 2.03	0.65 ± 0.91	1.56 ± 2.19	0.67 ± 0.63	2.78 ± 1.75	6.15 ± 4.00	2.27 ± 3.42	1.12 ± 1.12	14.09 ± 10.89	0.22 ± 0.25
Fg10695	FGSG_10695.3	1.04 ± 0.28	1.11 ± 0.48	1.48 ± 0.94	4.52 ± 1.41	2.50 ± 0.40	2.33 ± 0.09	2.67 ± 0.84	0.79 ± 0.46	0.64 ± 0.23	0.63 ± 0.17	0.87 ± 0.26	7.87 ± 1.77	1.76 ± 0.21	1.40 ± 0.35	1.07 ± 0.46	3.83 ± 0.61	1.14 ± 0.17
Fg10910	FGSG_10910.3	1.07 ± 0.41	1.02 ± 0.26	1.44 ± 0.06	2.81 ± 2.30	2.91 ± 0.17	2.17 ± 0.97	2.79 ± 0.98	0.71 ± 0.30	0.35 ± 0.16	1.05 ± 0.88	0.48 ± 0.02	5.60 ± 1.96	3.92 ± 0.10	4.33 ± 3.79	1.92 ± 0.12	2.60 ± 0.84	2.17 ± 0.74
Fg11389	FGSG_11389.3	1.08 ± 0.39	1.12 ± 0.58	0.95 ± 0.56	22.69 ± 10.27	2.93 ± 0.71	9.51 ± 2.68	3.88 ± 0.69	2.09 ± 0.87	1.33 ± 0.54	0.90 ± 0.64	1.61 ± 0.84	17.18 ± 1.06	4.75 ± 0.41	5.09 ± 1.31	0.84 ± 0.61	22.54 ± 9.22	0.89 ± 0.49
Fg11424	FGSG_11424.3	1.02 ± 0.19	1.09 ± 0.47	0.83 ± 0.47	5.48 ± 1.84	3.27 ± 1.02	11.67 ± 5.23	3.07 ± 0.67	1.06 ± 0.68	0.72 ± 0.25	0.60 ± 0.20	0.77 ± 0.39	26.36 ± 1.58	7.80 ± 1.12	1.90 ± 0.16	0.68 ± 0.22	4.12 ± 0.44	1.12 ± 0.73
Fg11282	FGSG_11282.3	1.00 ± 0.06	1.16 ± 0.63	0.63 ± 0.40	0.54 ± 0.42	1.13 ± 0.13	0.48 ± 1.07	1.82 ± 0.06	0.72 ± 0.33	0.63 ± 0.19	0.69 ± 0.25	1.39 ± 0.56	3.83 ± 0.25	2.12 ± 1.46	1.93 ± 1.66	0.69 ± 0.18	1.32 ± 0.42	1.50 ± 0.54
Fg11303	FGSG_11303.3	1.04 ± 0.29	1.08 ± 0.42	1.06 ± 0.86	8.00 ± 0.35	2.77 ± 0.86	3.01 ± 1.66	2.26 ± 0.88	0.94 ± 0.50	0.96 ± 0.54	0.51 ± 0.13	0.67 ± 0.30	12.84 ± 0.67	10.28 ± 1.35	7.57 ± 5.24	0.78 ± 0.31	5.95 ± 1.81	0.95 ± 0.50
Fg11038	FGSG_11038.3	1.02 ± 0.22	1.14 ± 0.56	0.97 ± 0.63	1.49 ± 0.24	2.09 ± 0.86	8.08 ± 3.77	0.77 ± 0.10	1.32 ± 1.40	0.61 ± 0.23	0.62 ± 0.33	0.78 ± 0.50	6.38 ± 1.23	1.29 ± 0.37	0.88 ± 0.06	0.82 ± 0.19	5.39 ± 1.60	0.61 ± 0.23
Fg11108	FGSG_11108.3	1.05 ± 0.33	1.16 ± 0.66	0.72 ± 0.56	5.64 ± 0.92	2.78 ± 0.13	3.45 ± 0.90	4.23 ± 1.71	2.16 ± 1.52	1.20 ± 0.36	0.75 ± 0.55	2.20 ± 0.69	18.13 ± 7.61	15.93 ± 3.53	3.35 ± 0.32	1.39 ± 0.78	22.13 ± 7.77	0.74 ± 0.45
Fg10960	FGSG_10960.3	1.01 ± 0.14	1.00 ± 0.07	0.70 ± 0.27	1.14 ± 1.18	1.02 ± 0.13	0.25 ± 0.12	4.73 ± 0.50	0.97 ± 0.43	0.90 ± 0.38	0.73 ± 0.25	1.18 ± 0.14	22.39 ± 4.44	1.85 ± 0.69	0.57 ± 0.08	0.78 ± 0.24	1.32 ± 0.31	0.63 ± 0.21
Fg10991	FGSG_10991.3	1.16 ± 0.54	1.05 ± 0.34	1.52 ± 1.24	8.47 ± 0.78	3.22 ± 2.03	2.31 ± 0.32	2.32 ± 0.61	1.00 ± 0.49	1.04 ± 0.67	0.49 ± 0.13	0.86 ± 0.31	10.27 ± 1.56	2.26 ± 0.52	2.42 ± 0.52	0.43 ± 0.18	4.73 ± 0.77	0.91 ± 0.80
Fg11002	FGSG_11002.3	1.06 ± 0.39	1.03 ± 0.27	0.87 ± 0.18	14.27 ± 11.91	10.29 ± 3.47	4.95 ± 2.87	5.09 ± 0.84	2.49 ± 1.22	1.51 ± 0.61	1.71 ± 0.84	2.83 ± 0.98	31.73 ± 7.02	8.87 ± 1.59	40.97 ± 27.82	1.38 ± 0.53	10.45 ± 0.94	1.24 ± 0.92
Fg11024	FGSG_11024.3	1.07 ± 0.42	1.01 ± 0.16	0.14 ± 0.05	0.34 ± 0.30	1.46 ± 0.46	1.14 ± 0.34	8.94 ± 0.40	0.67 ± 0.45	0.37 ± 0.25	1.13 ± 1.17	0.29 ± 0.02	18.41 ± 0.47	1.76 ± 0.54	2.56 ± 2.22	0.90 ± 0.25	1.05 ± 0.11	3.94 ± 1.16
Fg11465	FGSG_11465.3	1.08 ± 0.75	1.03 ± 0.25	0.15 ± 0.06	1.67 ± 0.40	0.86 ± 0.14	0.64 ± 0.47	0.20 ± 0.06	0.82 ± 0.58	0.32 ± 0.05	0.95 ± 0.69	0.43 ± 0.13	1.45 ± 0.36	1.50 ± 1.29	2.95 ± 1.33	1.86 ± 0.66	0.85 ± 0.44	1.63 ± 0.10

Fg11498	FGSG_11498.3	1.02 ± 0.20	1.04 ± 0.32	1.56 ± 0.51	1.83 ± 1.99	2.03 ± 0.07	1.81 ± 1.34	3.04 ± 0.03	0.81 ± 0.71	1.02 ± 0.45	0.75 ± 0.63	0.69 ± 0.04	0.68 ± 0.39	5.70 ± 2.81	1.78 ± 0.79	0.99 ± 0.33	4.05 ± 1.84	1.09 ± 0.06
Fg11536	FGSG_11536.3	1.07 ± 0.27	1.04 ± 0.32	0.58 ± 0.46	19.87 ± 12.60	6.97 ± 2.75	1.43 ± 1.30	2.36 ± 0.39	2.38 ± 1.21	1.51 ± 1.21	0.98 ± 0.44	2.49 ± 1.22	16.75 ± 2.79	12.40 ± 5.20	1.69 ± 0.31	0.66 ± 0.25	9.90 ± 2.93	0.96 ± 1.07
Fg10451	FGSG_10451.3	1.07 ± 0.42	1.18 ± 0.68	1.34 ± 1.06	21.61 ± 9.30	1.81 ± 0.82	2.47 ± 2.23	1.44 ± 0.97	0.88 ± 0.81	0.30 ± 0.08	0.65 ± 0.32	0.63 ± 0.58	5.59 ± 1.51	2.48 ± .2.71	2.25 ± 1.96	0.75 ± 0.20	2.46 ± 1.11	1.08 ± 0.49
Fg10461	FGSG_10461.3	1.03 ± 0.28	1.07 ± 0.44	0.94 ± 0.67	6.43 ± 4.83	3.39 ± 1.54	4.69 ± 0.71	4.56 ± 0.63	0.85 ± 0.65	0.72 ± 0.30	0.52 ± 0.06	1.21 ± 0.42	35.94 ± 2.41	4.05 ± 0.67	1.96 ± 0.26	0.58 ± 0.25	5.10 ± 0.63	0.96 ± 0.36
Fg09351	FGSG_09351.3	1.18 ± 0.69	1.04 ± 0.29	0.73 ± 0.26	1.49 ± 1.41	1.44 ± 0.56	2.23 ± 1.35	0.08 ± 0.01	0.75 ± 0.66	0.46 ± 0.13	2.89 ± 2.84	0.71 ± 0.45	0.65 ± 0.38	0.48 ± 0.14	0.46 ± 0.17	0.66 ± 0.05	0.22 ± 0.10	0.36 ± 0.12
Fg09195	FGSG_09195.3	1.06 ± 0.40	1.42 ± 0.97	1.08 ± 0.47	1.33 ± 1.11	2.70 ± 0.12	0.69 ± 0.50	2.53 ± 0.06	0.47 ± 0.19	0.30 ± 0.02	0.68 ± 0.58	0.51 ± 0.19	10.32 ± 0.15	1.83 ± 0.60	2.13 ± 1.49	0.92 ± 0.24	0.92 ± 0.30	4.12 ± 1.47
Fg09916	FGSG_09916.3	1.00 ± 0.10	1.03 ± 0.25	0.39 ± 0.16	1.95 ± 0.90	0.79 ± 0.26	0.79 ± 0.48	5.37 ± 0.19	0.49 ± 0.19	0.45 ± 0.17	0.68 ± 0.57	0.61 ± 0.04	5.37 ± 0.35	1.61 ± 1.01	1.11 ± 0.11	0.63 ± 0.05	3.17 ± 2.09	0.77 ± 0.29
Fg09671	FGSG_09671.3	1.04 ± 0.28	1.14 ± 0.47	0.30 ± 0.03	4.02 ± 0.13	1.66 ± 0.52	0.59 ± 0.02	1.74 ± 0.26	0.67 ± 0.41	0.61 ± 0.18	0.38 ± 0.12	0.55 ± 0.24	8.46 ± 0.75	1.78 ± 0.19	1.80 ± 0.37	0.48 ± 0.03	5.28 ± 2.94	0.93 ± 0.46
Fg11655	FGSG_11655.3	1.00 ± 0.07	1.15 ± 0.61	1.24 ± 1.06	2.20 ± 2.05	2.01 ± 0.16	1.52 ± 1.22	7.02 ± 0.34	1.03 ± 0.23	0.79 ± 0.12	1.80 ± 0.17	0.89 ± 0.22	28.99 ± 1.60	1.90 ± 0.15	2.31 ± 1.34	5.79 ± 1.81	5.41 ± 2.02	6.36 ± 2.44
Fg12534	FGSG_12534.3	1.00 ± 0.07	1.04 ± 0.28	1.41 ± 0.55	0.83 ± 0.69	3.95 ± 0.12	1.36 ± 0.48	0.88 ± 0.30	1.05 ± 0.58	1.18 ± 0.11	1.58 ± 1.08	1.18 ± 0.06	0.96 ± 0.37	3.66 ± 1.05	1.13 ± 0.07	2.48 ± 0.15	0.60 ± 0.02	0.48 ± 0.03
Fg12568	FGSG_12568.3	1.21 ± 0.28	1.09 ± 0.45	0.94 ± 0.79	6.78 ± 1.62	2.53 ± 0.84	1.94 ± 1.29	1.90 ± 0.66	0.88 ± 0.52	0.87 ± 0.38	0.53 ± 0.12	0.94 ± 0.32	12.43 ± 1.75	4.81 ± 0.26	2.04 ± 0.36	0.80 ± 0.27	4.99 ± 0.46	0.87 ± 0.40
Fg08809	FGSG_08809.3	1.08 ± 0.44	1.02 ± 0.22	0.53 ± 0.20	1.48 ± 1.03	0.97 ± 0.37	3.97 ± 2.00	2.73 ± 0.89	0.58 ± 0.39	0.32 ± 0.13	0.33 ± 0.12	0.69 ± 0.23	7.75 ± 0.53	0.57 ± 0.35	2.07 ± 1.82	0.69 ± 0.19	1.70 ± 0.17	0.55 ± 0.21
Fg13176	FGSG_13176.3	1.00 ± 0.11	1.14 ± 0.58	0.99 ± 0.61	2.65 ± 0.57	1.02 ± 0.16	48.59 ± 17.39	0.83 ± 0.36	0.89 ± 0.76	0.29 ± 0.09	1.10 ± 0.72	0.66 ± 0.22	3.38 ± 1.66	1.82 ± 0.19	0.43 ± 0.04	1.25 ± 0.48	1.32 ± 0.60	0.88 ± 0.52
Fg12737	FGSG_12737.3	1.00 ± 0.05	1.17 ± 0.66	1.49 ± 0.53	1.19 ± 1.20	2.00 ± 0.14	0.46 ± 0.42	1.35 ± 0.44	0.60 ± 0.43	0.33 ± 0.12	4.02 ± 1.02	0.47 ± 0.03	0.97 ± 0.10	0.49 ± 0.05	2.82 ± 2.79	2.26 ± 1.39	0.69 ± 0.27	1.03 ± 0.06
Fg12599	FGSG_12599.3	1.29 ± 0.52	1.13 ± 0.57	0.95 ± 0.67	22.20 ± 15.79	18.20 ± 5.31	1.17 ± 1.15	3.57 ± 0.57	1.60 ± 0.39	2.14 ± 1.86	1.77 ± 0.59	4.21 ± 0.76	24.47 ± 14.06	14.61 ± 2.55	2.07 ± 0.20	1.84 ± 0.68	6.52 ± 1.88	1.84 ± 1.54
Fg13445	FGSG_13445.3	1.00 ± 0.06	1.12 ± 0.49	1.20 ± 0.90	2.32 ± 1.19	4.51 ± 1.56	1.77 ± 0.15	3.23 ± 1.42	1.68 ± 1.03	0.89 ± 0.32	0.57 ± 0.25	1.79 ± 1.25	12.53 ± 1.71	4.98 ± 0.89	1.68 ± 0.63	0.85 ± 0.36	8.92 ± 2.97	1.17 ± 0.71
Fg13459	FGSG_13459.3	1.00 ± 0.11	1.10 ± 0.25	1.09 ± 0.97	15.07 ± 1.11	1.93 ± 0.36	10.07 ± 6.05	2.77 ± 1.02	1.22 ± 0.53	0.78 ± 0.24	0.44 ± 0.15	1.01 ± 0.20	14.48 ± 1.73	1.82 ± 0.60	3.99 ± 0.65	0.66 ± 0.38	6.22 ± 1.45	1.33 ± 1.48
Fg14007	FGSG_14007.3	1.03 ± 0.25	1.15 ± 0.63	1.55 ± 1.06	26.58 ± 19.56	3.19 ± 1.42	8.12 ± 3.46	2.27 ± 0.93	1.14 ± 0.45	1.42 ± 0.84	1.58 ± 1.08	1.41 ± 0.40	27.55 ± 11.69	18.77 ± 10.99	2.64 ± 1.35	0.52 ± 0.28	8.42 ± 0.42	0.68 ± 0.31
Fg13739	FGSG_13739.3	1.03 ± 0.24	1.20 ± 0.84	1.10 ± 0.92	34.47 ± 3.60	2.81 ± 1.30	2.00 ± 0.65	2.58 ± 0.65	2.04 ± 0.98	2.22 ± 1.68	1.11 ± 0.44	2.35 ± 0.57	51.28 ± 16.15	5.70 ± 0.85	3.60 ± 2.32	0.93 ± 0.52	13.25 ± 4.80	0.51 ± 0.09
Fg13796	FGSG_13796.3	1.34 ± 0.75	1.59 ± 1.23	0.36 ± 0.15	3.80 ± 0.77	3.10 ± 2.5	2.55 ± 1.89	0.93 ± 0.35	1.95 ± 1.97	0.68 ± 0.08	1.74 ± 1.14	0.47 ± 0.33	5.68 ± 1.82	2.95 ± 1.05	0.47 ± 0.34	0.90 ± 0.41	3.46 ± 2.19	1.49 ± 0.97
Fg13797	FGSG_13797.3	1.14 ± 0.60	1.13 ± 0.67	0.30 ± 0.13	2.09 ± 1.34	0.58 ± 0.20	1.08 ± 0.76	0.42 ± 0.07	1.52 ± 1.49	0.37 ± 0.06	1.10 ± 0.39	0.24 ± 1.11	1.43 ± 0.32	0.81 ± 0.47	0.37 ± 0.21	0.26 ± 0.13	1.73 ± 0.93	0.39 ± 0.02

DISCUSSION

The construction of genome-wide knockout mutant libraries with corresponding phenotypic datasets in model organisms has led to great progresses in basic and applied biology. P450 enzymes play important roles in diverse types of primary and secondary metabolism and the degradation of xenobiotics in eukaryotic organisms (van den Brink et al., 1998). Plant pathogenic fungi possess a relatively large number of P450 genes compared with other eukaryotic organisms (Črešnar and Petrič, 2011), suggesting that P450-mediated molecular processes are particularly important for these fungal species. However, no systematic functional analysis of P450s has yet been attempted in filamentous fungi, including plant pathogens. In this study, I targeted each of 119 putative P450s for gene deletion and generated a comprehensive phenome of 102 P450s related to 38 traits in the plant pathogenic ascomycete *F. graminearum*. In most cases, the P450 mutants with mutant phenotypes showed a single phenotypic defect, demonstrating that my genetic resources are valuable for further genetic studies on specific fungal developmental processes, including sexual development and virulence. In particular, genes specifically involved in virulence (5), conidiation (1), and sexual development (2) have rarely been found in *F. graminearum*.

There has been considerable interest in identifying factors involved in reproduction in *F. graminearum* because this fungus utilizes sexual (ascospores) and asexual (conidia) spores for primary and secondary infection, respectively (Leslie and Summerell, 2006). Some P450 enzymes have been reported to regulate

development by producing phytohormones in plants (Kushiro et al., 2004). In *Aspergillus nidulans*, the hormone-like fatty acid-derived oxylipins serve as signal molecules that modulate reproductive processes by affecting the timing and balance of asexual and sexual development (Fischer and Keller, 2016). *CYPA* orthologs encoding elongationless 2 are involved in sexual development in the mushroom-forming fungi *Coprinopsis cinerea* and *Agaricus bisporus* (De Groot et al., 1997; Kamada, 2002). Based on expression profiles, P450s appear to have distinct functions in sexual and asexual reproduction processes in *F. graminearum* (Fig. 2).

I discovered that sex-induced *Fg08320* and *Fg09671* are required for selective perithecium maturation and initial perithecium formation, respectively, in *F. graminearum*. The Δ*Fg09671* strains failed to switch its developmental stage from mycelial growth to perithecium production, and the maturation of overproduced small perithecia was arrested in the Δ*Fg08320* strains; in contrast, in the wild-type strain, mycelial growth was completely inhibited and perithecia were selectively matured among the initial perithecia after sexual induction (Fig. 5). I suspect that *Fg08320* and *Fg09671*-mediated signaling molecules are involved in each step of perithecium production in *F. graminearum*. Likewise, *Fg06068* is required for phialide formation and function in this fungus (Fig. 4). Further studies are necessary to identify the P450-mediated signaling molecules to elucidate the molecular mechanisms underlying fungal reproduction processes.

Plants synthesize and accumulate plant antibiotics known as phytoalexins that

are toxic to most fungal pathogens (Paxton, 1980). However, fungi frequently possess the ability to detoxify plant toxins using P450s. Pisatin demethylase (*PDA*, CYP57A1) of *Nectria haematococca* is a well-known P450 enzyme that degrades pisatin, a fungistatic isoflavonoid pterocarpan produced by host plants (George et al., 1998). Introduction of the *PDA* (*FgPDA1*) ortholog into a *PDA*-deficient *F. oxysporum* strain was shown to promote pathogenicity in pea (Coleman et al., 2011); however, orthologs of *PDA* are unclear in *F. graminearum*. Five P450 mutants (*Fg03700*, *Fg02111*, *Fg00012*, *Fg10451*, and *Fg12737*) were revealed to be specifically defective in virulence (Supporting information Table S1 and Fig. 6), and two of these genes (*Fg03700* and *Fg12737*) are highly upregulated during plant infection (Harris et al., 2016). Moreover, all of these genes were markedly upregulated under at least one xenobiotic condition compared with the untreated control, suggesting that they function in the detoxification of plant toxins in *F. graminearum* (Fig. 10). Because few virulence-specific mutants of *F. graminearum* have been available, further studies addressing these mutants will be helpful for understanding host-pathogen interaction mechanisms.

Azole fungicides comprise a large number of commercially available fungicides, and more than 36 azole drugs have been used in agricultural applications. These compounds specifically bind to the sterol 14α-demethylase encoded by the *CYP51* gene and therefore inhibit the biosynthesis of ergosterol, which is the principal sterol present in fungal membranes. In *F. graminearum*, single or double deletion of three *CYP51* paralogs (*CYP51A*, *CYP51B*, and

CYP51C) was not found to affect fungal development or ergosterol contents (Liu et al., 2011; Fan et al., 2013), suggesting that *CYP51* genes have redundant functions, and additional P450s are involved in ergosterol biosynthesis. Likewise, 17 P450 mutants showing altered sensitivity to azole drugs are expected to be closely related to ergosterol production in *F. graminearum* either directly or indirectly (Supporting information Table S3). Because the ergosterol biosynthetic pathway is still a good target for fungicides, elucidating the novel mechanisms of these P450s will provide genetic resources for the development or application of new fungicides.

Several filamentous fungi are able to detoxify environmental pollutants via substrate-inducible P450-mediated reactions (Cerniglia et al., 1978; Sutherland, 1992; Bezalel et al., 1997). For example, in the white rot fungus *Phanerochaete chrysosporium*, P450 enzymes show an extraordinary ability to degrade a broad spectrum of aromatic, alicyclic, and aliphatic chemicals (Reddy, 1995). In my study, the transcript levels of most P450 genes were markedly upregulated under at least one xenobiotic condition (Fig. 10). In particular, many P450 genes were highly induced in the presence of 1-naphthol (94 genes), naphthalene (68 genes), 1-dodecanol (75 genes), and 4-octylphenol (76 genes), but only one mutant (Δ*Fg01972*) showed reduced growth in the 1-dodecanol-supplemented condition, demonstrating that most P450s exhibit overlapping biochemical functions in *F. graminearum*. However, P450 enzymes may not be the only source of tolerance to xenobiotics. Not only P450s but also ATP-binding cassette (ABC) transporters,

major facilitator superfamily (MFS) transporters and other enzymes such as peroxidase have been shown to play important roles in xenobiotic detoxification in *P. chrysosporium* and *C. albicans* (Bumpus and Aust, 1987; Vandeputte et al., 2012). Thus, the requirements for P450s in fungal development are not properly reflected by developmental phenotypes, likely because of their redundant functions among P450s and the existence of other mechanisms (Sanglard et al., 1998).

In conclusion, I constructed a *F. graminearum* P450 mutant library and a comprehensive phenotypic dataset. Most P450s have redundant functions in xenobiotic detoxification and fungal development, suggesting that other approaches, such as RNA silencing, are required for genetic studies on P450s in filamentous fungi. Nevertheless, I identified novel P450s that are specifically involved in fungal reproduction and virulence. This study is the first systemic functional analysis of P450s, and my results provide a platform for further metabolomic and biochemical studies on P450s in filamentous fungi, including plant pathogens.

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e45432.

APPENDIX

Table S1. Primers used in this study.

A. Primer for transformation

Gene name	Oligo name	Sequence
Fg07859	07859.3-5F	CATTGCGAATACCCAGGGAAGTC
	07859.3-5R	gcacaggtacacttgtttagagTGACTTCAAAGGCTACTACCAGGGC
	07859.3-3F	ccttcaatatcatcttctgtcgACAACCACCATTCTGAGCGTGAGT
	07859.3-3R	TCTTGCTTAACCTCGCCACCCT
	07859.3-5N	CACCTGCGCCTGTGAACTTGAT
	07859.3-3N	GCTTTGCCCAACGTTCCTGAG
Fg07804	07804.3-5F	CATGAGCCTCTTGGAGTGGTCG
	07804.3-5R	gcacaggtacacttgtttagagCCAGCCATGCGTGAGTAGGAGA
	07804.3-3F	ccttcaatatcatcttctgtcgTATGGCGCGGCGAATAGAGAC
	07804.3-3R	CCAGCCATGCGTGAGTAGGAGA
	07804.3-5N	CTGGTAAAGTGCTGGCAACAACG
	07804.3-3N	AGCGGGTATAAGGATCAAATGCG
Fg08079	08079.3-5F	GCTGTATGCCATCCGTCCTACCT
	08079.3-5R	gcacaggtacacttgtttagagGCCAGATCAAGGGAAGGACAGTG
	08079.3-3F	ccttcaatatcatcttctgtcgCGATGGAGATTTTGGAGGGCTT
	08079.3-3R	CCCCGACTCAGACGCCTACAC
	08079.3-5N	TGGGCACAGCGGGACACT
	08079.3-3N	ACCAAGTCCCCGAAATACCCAGTT
Fg04717	04717.3-5F	CCATCAGAACTGATCCCAAGACCA
	04717.3-5R	gcacaggtacacttgtttagagTAACGGACCTTCACAATGCGAGA
	04717.3-3F	ccttcaatatcatcttctgtcgACTTTGTGGCCATACTTTCTCCCC
	04717.3-3R	CCCTAGATCCCTCGGTCTGACTTC
	04717.3-5N	CGAGAGTGTCATCCGAGCCGTA
	04717.3-3N	GGATCACGGCAGAATATGAACAGG
Fg01868	01868.3-5F	ACAAGAACGGGGACGTAATGAGTG
	01868.3-5R	gcacaggtacacttgtttagagAAGTTTTGCGAATATGGAATGCGAT

B. Primer for qRT-PCR

Name	Oligo name	Sequence
Fg07859 FGSG_07859.3	RT1-F	TGGCCCATCGGTCCAAGTCT
	RT1-R	TGTACCGCTTTTGGTTGGAATGAT
Fg07804 FGSG_07804.3	RT2-F	GTTTCGCTCGTGCTCTTCATCAG
	RT2-R	TCTGCGGGAATAGGCCTTCTTT
Fg08079 FGSG_08079.3	RT3-F	TCACACAGTTTTGCACCACAGTCC
	RT3-R	ATCGCCGATGATATCAAGGACAAG
Fg04717 FGSG_04717.3	RT4-F	CCGACTCAAAAGAATGGCACTCA
	RT4-R	AGAAACCAATGGATATAAGGGCGAA
Fg01868 FGSG_01868.3	RT6-F	TCAACCGCGACGAGAAGTGG
	RT6-R	CCATGTTTTGCAGTCGCTCCTT
Fg07596 FGSG_07596.3	RT7-F	CGGTAACGGTATGCGAGGATGTAT
	RT7-R	ACCTTTCGGTCGTTGCGGAG
Fg05960 FGSG_05960.3	RT8-F	AGCTTGCATGTCCATCCCTCTG
	RT8-R	GCTCCTGCTCGACTCGTTTGTAA
Fg03860 FGSG_03860.3	RT9-F	ACCAAAGATGAGGGAGGCAAATG
	RT9-R	TCGCTGAGGATGGCTTGACTTC
Fg07765 FGSG_07765.3	RT10-F	TGGAGATCTGGCCTTTGGTGAG
	RT10-R	TACCTTTTCCCTCGCCATTTCC
Fg02982 FGSG_02982.3	RT11-F	TCAAACCGACCCTGTGAAGCA
	RT11-R	CCAGCATTATGTCCAGTAAACCGC
Fg03264 FGSG_03264.3	RT12-F	TGACTGGCTTGACAACAGAGTGG
	RT12-R	GGCGTCAGGGCTAGTGAGAAGAT
Fg03260 FGSG_03260.3	RT13-F	AGAATCTCAAACATCCCCCAAACC
	RT13-R	AGGTGGTAGAAAAGAGCAGAAAGGC
Fg05806 FGSG_05806.3	RT14-F	ACGCTCTTGAATGCCGATAATGTT
	RT14-R	ATCCGATCCCGCAATAATAAGGTT

	01868.3-3F	ccttcaatatcatcttctgtcgACGAGCACCAATACTTTGATACCCAG
	01868.3-3R	ATCCTGAGCAATCAACCAACTCCA
	01868.3-5N	TCACTCTTCCGCAAAACTCCCA
	01868.3-3N	ACATTGCCATTGAAAGAATACCGTCT
Fg07596	07596.3-5F	CCGGTTTTATCACCCCATTACTCAG
	07596.3-5R	gcacaggtacacttgtttagagTGTCGAGTTGTGTTGTGGAGTGTTG
	07596.3-3F	ccttcaatatcatcttctgtcgCCAAGATGCGATGCCAGTCCT
	07596.3-3R	CTCGGCACACGTAGATTCACTCTTC
	07596.3-5N	CTCTCGTCGTCGCGGGTTTAG
	07596.3-3N	CGCACTCATGTTACTACGACTTACCG
Fg05960	05960.3-5F	GCCAGACCAAGCAGTATCAGCAAC
	05960.3-5R	gcacaggtacacttgtttagagCAAGTTCAATTGGCGCATAGGC
	05960.3-3F	ccttcaatatcatcttctgtcgGAAAAGGTTCCGTGGGCTACTCTC
	05960.3-3R	GGCTGAATGCATCACATTGGAAA
	05960.3-5N	TTCATCCATCGCCACTTCTTCAA
	05960.3-3N	GGACGGGATAACTGCTTGTAGGTTC
Fg03860	03860.3-5F	AAGAAGAAGAAGCATTGCGTGAGC
	03860.3-5R	gcacaggtacacttgtttagagTGACGCGACGGCTGTTCTTTA
	03860.3-3F	ccttcaatatcatcttctgtcgCCTGGCCTAGTCCCTACATAGCG
	03860.3-3R	TCCAGAGGGCCAACCAGAGACT
	03860.3-5N	TCACAGAGAGATGCAGCGACACA
	03860.3-3N	TCTCACACCAGTCAGGGACACAAA
Fg07765	07765.3-5F	CGGTCTGGGTCTCATATCTGGTCTC
	07765.3-5R	gcacaggtacacttgtttagagGATCCGGATCTTGGACATCTTGC
	07765.3-3F	ccttcaatatcatcttctgtcgTCTTTTGTTAAACGAGGGACCAGTGT
	07765.3-3R	ACGGTGAATTTGGCAACAGTGTAAG
	07765.3-5N	AGATGGCTTCGTATGGTCTTGGTG
	07765.3-3N	TGACTCTCTCGGGATGAGCAGGT
Fg02982	02982.3-5F	CAAGCCAGGGTGACTTACACAAGAA
	02982.3-5R	gcacaggtacacttgtttagagCGAGATGGGATGTGCGTGTTTT
	02982.3-3F	ccttcaatatcatcttctgtcgGTTGGTCAGAGAACATCACAGAGCCT

CYP51A FGSG_04092.3	RT15-F	CCACTGCCTCACAACCGAAAAC
	RT15-R	GCTGGCCTGCCATGAGTAGAGTAA
Fg01284 FGSG_01284.3	RT16-F	CCTGGGATGGGATGTCAAGTCTC
	RT16-R	CAACGCTCGCTCGATGTAAAAGT
Fg03191 FGSG_03191.3	RT17-F	GGAGGGCAAGGGAATTACTGTCA
	RT17-R	GCGAGACGTTCGAATGTGATGTC
Fg03700 FGSG_03700.3	RT19-F	CGAAGCTGCCGCCATTATTCT
	RT19-R	GCTAGTACCGGGGAACCAGTCAG
Fg03686 FGSG_03686.3	RT20-F	ACCGAATACCGCCGAGGACTTA
	RT20-R	AAGAAGGTTCGACAAGAGAGTGCG
Fg02138 FGSG_02138.3	RT21-F	GAGCGTATCATGGAACCCCTTGT
	RT21-R	GCGGTGATCTGGGTGTTTTCG
Fg02118 FGSG_02118.3	RT22-F	CCGCCACATCAAGGAACTCTACC
	RT22-R	GTCCTCGATGCTGGGGTCTGA
Fg02117 FGSG_02117.3	RT23-F	ACAAAGCCTCTGTGTCGAAAATGG
	RT23-R	CAGGGTGCTCGTAGATGGTTGG
Fg02114 FGSG_02114.3	RT24-F	CTCACGGAGTTGTTTGTTGGTTTG
	RT24-R	CTCCCAAAAGACATCGGCAAAAT
Fg02111 FGSG_02111.3	RT26-F	AACCCGTCCCTGATTTCCACA
	RT26-R	CGACGGATGCCAAGATTAGATAGC
Fg02458 FGSG_02458.3	RT27-F	AGGTCGAACTCAATGAATGGATGC
	RT27-R	AGCTTGGCAGCCAGAGGAAATAGT
Fg05334 FGSG_05334.3	RT28-F	TGCCGAGAAGGACCCCAGTAA
	RT28-R	GGACAACATCGTCTGTGAAGCGT
Fg02672 FGSG_02672.3	RT29-F	GTCGATCGAATACACCGTCCAACT
	RT29-R	GCGATGGTCGTCCTTCAGCA
Fg02668 FGSG_02668.3	RT30-F	GCCTGATCCTGGTTTGCTCTTTG
	RT30-R	ACAAAGGCGCAAGATCAAGGTATG
Fg02929 FGSG_02929.3	RT31-F	TTGGCTACAAGGCGCAGTATTTT
	RT31-R	TCCTTTTCCGACGGTAGCATAGAT
Fg02872 FGSG_02872.3	RT32-F	CGTGCACTTCTTGGCCTGTATCT

	02982.3-3R	TCCTCGGATGTCTAGTATGCGGTATC
	02982.3-5N	CACTTCTCGACAACAATCCGTAACAG
	02982.3-3N	TGGCTAGGAGTACAGAAATGAGAGTCG
Fg03264	03264.3-5F	AGACTTGAAACCAGCACTGGAACATT
	03264.3-5R	gcacaggtacacttgtttagagCTCGCCATGTCAGCACATTCTTT
	03264.3-3F	ccttcaatatcatcttctgtcgCTTGCTGGATCAAATTGTCGTGG
	03264.3-3R	CAGTTTGATGTCAGTCGTAGTAGCCG
	03264.3-5N	AAAGGAATTGGAAGGGATGTTACGA
	03264.3-3N	TCTAGGAAGAGGCTCGCATCATTATG
Fg03260	03260.3-5F	AATTTGTCAAGATGCTTTCAAGTCGC
	03260.3-5R	gcacaggtacacttgtttagagCAGACGTTTTCGGAATGGATACAGA
	03260.3-3F	ccttcaatatcatcttctgtcgTTCGTTTGCCCGGGAACATTA
	03260.3-3R	GCCTGCCTGCATCTTGTTTCTCT
	03260.3-5N	TCTGAAGACCAAGGAGGAGCTAAAAAT
	03260.3-3N	CACCTACCAGGTCCCATTCTCATTC
Fg05806	05806.3-5F	CTATCATGCCATCAGATCCCGAAC
	05806.3-5R	gcacaggtacacttgtttagagCCACATCCCGATTTACCCCATT
	05806.3-3F	ccttcaatatcatcttctgtcgGTTCCCCAATCCTCTCAAGCATCT
	05806.3-3R	GTTACAGGGCCAATCACAAGGTCA
	05806.3-5N	GTTTGTTGCCTTTCCTGAAGTTTGTAA
	05806.3-3N	GGCTGCCTACTCAGAGTTTTCCG
CYP51A	04092.3-5F	ACCATCAAATGAACGCAGGGG
	04092.3-5R	gcacaggtacacttgtttagagGGAAGGCAACTACTCAAGCAGGG
	04092.3-3F	ccttcaatatcatcttctgtcgGAGTCAAGTTGTGTTAGCATCCCGAT
	04092.3-3R	GGGCTTTGCAGGGAGGTGTATT
	04092.3-5N	TTGAAGTTGTGCAGGATGTGGGTA
	04092.3-3N	ATGCTTCCATGTCATGACTCCCAC
Fg01284	01284.3-5F	TCTGGGCAAAGACGTTGTGGTC
	01284.3-5R	gcacaggtacacttgtttagagCTATGAACCCACGGACGTCAAGAG
	01284.3-3F	ccttcaatatcatcttctgtcgGATGCTGCAGTCTCGTGGTGTCT
	01284.3-3R	CTCGTCCAGCACACTATGACACCTC

	RT32-R	TGGAACCAAAGGGATCAGTGTCA
Fg03548 FGSG_03548.3	RT33-F	GTTCCCGGCCATCCTTTACATC
	RT33-R	TAGTGGGTGCATAGTGCCTGACAG
Fg03542 FGSG_03542.3	RT34-F	AGCTACTTTGCGCGAATCCTCC
	RT34-R	AAACACTAGGTCCATGGGCAAGAA
TRI11 FGSG_03540.3	RT35-F	GCCCAGGCCATGCTTGAAC
	RT35-R	GACTCTCAAAGGCCAGAGCAACC
TRI4 FGSG_03535.3	RT36-F	GTTCTCGTCGCGGCTACCTGA
	RT36-R	TCGTTGTGCTTGCCATAGAATCG
Fg03498 FGSG_03498.3	RT37-F	TAGAGTGGGTTAGGTACGCAGTCG
	RT37-R	CGTCGGTAGGGATATTCGTGTTGT
Fg06068 FGSG_06068.3	RT38-F	CAACAAGGCCCTCAAGGACATCTA
	RT38-R	GGATGTATCGCTGCATCTCTTTCA
Fg01786 FGSG_01786.3	RT39-F	TTCACGCAATCAAAGCCAATCTC
	RT39-R	GTCGAAGCAAGCCAGAACATAACC
Fg01767 FGSG_01767.3	RT40-F	AGCACGCTACAGCCAAGAAGATG
	RT40-R	TTTGATAGACGCGCGGTGATG
Fg01745 FGSG_01745.3	RT41-F	TTGCCAAAAAGTCCGAGGTCTG
	RT41-R	CCCCACTGAGAAAGCTTGAGGTAG
Fg01740 FGSG_01740.3	RT42-F	AATCGCTTCGTCACGCACTACC
	RT42-R	CTCATAAAATCACGACGACCTGGG
Fg01739 FGSG_01739.3	RT43-F	GTTCATCCCCGAGAGTGTCATCAT
	RT43-R	ACCACTCTCGTGGACAAGGAAGC
Fg01722 FGSG_01722.3	RT44-F	GACGACGACGCCTGAATCCAT
	RT44-R	CGAGTTGACCGAGTGGCTTCC
Fg05113 FGSG_05113.3	RT45-F	GTACCCTTTGCCGCTGCTCA
	RT45-R	AGCTCGTCTTGTGCCTTCTTTTGT
Fg01583 FGSG_01583.3	RT46-F	CACAAATCTGCGCTCAACTTCAAA
	RT46-R	CTACGGCTAGTGTGTACGCTGTGG
Fg01048 FGSG_01048.3	RT48-F	TTGCTATGCTACCGTGGCTTCTTT
	RT48-R	ACGTTGCATCTTGTGCTCATCG

	01284.3-5N	GAGTGCTTCGCCGGTTCAGAT
	01284.3-3N	ACACTTAAACAAGCAGCTCGCCATA
Fg03191	03191.3-5F	CGACATTTGCAGTTCCGCTACG
	03191.3-5R	gcacaggtacacttgtttagagAACAACGGATATTGCAGTGAAGGG
	03191.3-3F	ccttcaatatcatcttctgtcgATGCATGTTCACGTGGACTGGTAA
	03191.3-3R	TCCGCATACTAGTCGAGCACATTG
	03191.3-5N	ACAACGACACTGGTGATACCGAAAG
	03191.3-3N	GACCCGGCGTTAGTCAGAGACAT
Fg03700	03700.3-5F	CGACGCCATCTTCGCCTAAATA
	03700.3-5R	gcacaggtacacttgtttagagCACAGCCGGCAGAAGGAGTAA
	03700.3-3F	ccttcaatatcatcttctgtcgCTTCGTTGCCCAAGGATTCTCT
	03700.3-3R	TGGTCCGTCATCCACATTTCATC
	03700.3-5N	ATAAGCGCGGGCAATAAATGACT
	03700.3-3N	GACCACGCTTATCAACGCTATCG
Fg03686	03686.3-5F	GGCTTCCAATCCTTTCATCGTGT
	03686.3-5R	gcacaggtacacttgtttagagAAAGATGGATGACCCCGAGAGAAG
	03686.3-3F	ccttcaatatcatcttctgtcgAAGCTGAGCTACAAACTCCGCCTAT
	03686.3-3R	CCCCTCCTCCTCCCAGTGTTC
	03686.3-5N	TGCAGTGGCTCTTGTCATCATCAT
	03686.3-3N	TGCTGCCTCCATCAACTATCGC
Fg02138	02138.3-5F	TGGCGTAGACGAGGCTGATTTG
	02138.3-5R	gcacaggtacacttgtttagagAGTCGCTGGCTGGTCTACTGGTC
	02138.3-3F	ccttcaatatcatcttctgtcgGATGAGCTGTGATTGGCTGAGTGA
	02138.3-3R	TCGAACTTACGGAAACGCTCAAAG
	02138.3-5N	GGGCATCTTCTTGTGAGGAGCATA
	02138.3-3N	CTATCTCACGCTCGACCTGACCC
Fg02118	02118.3-5F	TCAAGAAACGCAAACATGGAGACA
	02118.3-5R	gcacaggtacacttgtttagagTGTGGATGAGGAGGGAATGGAAT
	02118.3-3F	ccttcaatatcatcttctgtcgGAGATGCATGAAGCAAGGGTAGAGAG
	02118.3-3R	TGACGTTACACATCTTCACCGCTG
	02118.3-5N	TCCTGGAGATACTCGCCTCTGATG

Fg02419 FGSG_02419.3	RT49-F	CATTCGGCTTTTTGGAGACAGACT
	RT49-R	CAGGACCAAAGCGTTCAGCAGTA
Fg02371 FGSG_02371.3	RT50-F	TCTCTGACGCATCTCGCAACTG
	RT50-R	TTTCCGCAATAAGTTCATCGTCAGT
Fg02367 FGSG_02367.3	RT51-F	CACAAATCTGCGCTCAACTTCAAA
	RT51-R	CTACGGCTAGTGTGTACGCTGTGG
Fg02366 FGSG_02366.3	RT52-F	AGGTGCCATTGCGCTTCTTGT
	RT52-R	CGGTCTTGGGCCCTTGAATG
Fg02344 FGSG_02344.3	RT53-F	TCCGGGACTTTTCGCAGATG
	RT53-R	CCCAAGTAGCCAGGTTAGGTTCC
Fg03796 FGSG_03796.3	RT54-F	GAACGCAAATGCCCTCAACTACA
	RT54-R	CGGGGAAGATGTTAACCAGTAGCA
Fg03741 FGSG_03741.3	RT55-F	TGATGACTGCGAGAAAGGGACAA
	RT55-R	GGGTACGTAATCCTGCCAGTTGTT
Fg00863 FGSG_00863.3	RT56-F	TGCTCTCGTTGCTCAGGCTATTG
	RT56-R	GAGGTCATCAACTCGGTCAGGCT
Fg00012 FGSG_00012.3	RT57-F	CCGAGACGACTGATGGCACAC
	RT57-R	TCAGTCTGTTTTTGGCTTGGTGGT
CLM2 FGSG_00007.3	RT58-F	CCTCCACAACCCAGAAACCTATCA
	RT58-R	ATCCTTTTCGATGTCAAATGCTGC
TRI1 FGSG_00071.3	RT59-F	CAACCCTGCTGCTACCCAACTATT
	RT59-R	CGCTGTCGAGAAGGAACATCTTG
Fg00127 FGSG_00127.3	RT60-F	GGCCACGAAACTACCACCAATGT
	RT60-R	CGTTGAGCTTCATCCCGCATAG
Fg08412 FGSG_08412.3	RT61-F	TTCGACTACCGCGCTTTCACA
	RT61-R	GCAAGGATAGACTGGGCACGAG
Fg08377 FGSG_08377.3	RT62-F	CAGGCTGTGGTCCATGAGGTTAC
	RT62-R	CGCATTCGAGCTCCCGTAAA
Fg08320 FGSG_08320.3	RT63-F	AGGTGCCATTGCGCTTCTTGT
	RT63-R	CGGTCTTGGGCCCTTGAATG
Fg08207 FGSG_08207.3	RT65-F	CCTCAAGCACGTTTCACAGCAC

	02118.3-3N	TCCCTCAGTCTTGAGCAAGTGTCC
Fg02117	02117.3-5F	AATATCCAAGTGAGCCAAGCGATG
	02117.3-5R	gcacaggtacacttgtttagagGCGTTTCTTGATGCTGGGAATAGA
	02117.3-3F	ccttcaatatcatcttctgtcgTTAGCCTGTAGCTTTGCCACTTGAA
	02117.3-3R	GGCTTAGCAACAATCCCATCAGTG
	02117.3-5N	TGATAAAACAATGCGCATAAGAGGC
	02117.3-3N	GGTAGTGTCCCTGAGAGAATCCCG
Fg02114	02114.3-5F	GCGTGTGCCCAGGTAAAAAGTTC
	02114.3-5R	gcacaggtacacttgtttagagAGAGGTTTCGAGCCAGTGATTCA
	02114.3-3F	ccttcaatatcatcttctgtcgAGGGATATGGTTGTTCGGTTATGG
	02114.3-3R	AAACCTCCGTACTTCTGGCAAAGA
	02114.3-5N	AGGGAGAGGTGCTATTGTGGAGTG
	02114.3-3N	CACTACAACCATGACGGCTCAGAC
Fg02111	02111.3-5F	GCCCTGGTGCCAAAATGTTCTAC
	02111.3-5R	gcacaggtacacttgtttagagCGTTCTTTGACATATGGTGGTTCC
	02111.3-3F	ccttcaatatcatcttctgtcgCAAGACGCAGTGGTTGGGAGA
	02111.3-3R	TTGCTACGAAAAGGAATGTTGGTG
	02111.3-5N	GGGACATAGCTTGGATCACGAGAG
	02111.3-3N	TGTGGAACGTCCTCAGGGTATCA
Fg02458	02458.3-5F	CCTATGACGGCCCAGATTTACTTC
	02458.3-5R	gcacaggtacacttgtttagagGAGTCATCATCCAGGCAAAGAAAA
	02458.3-3F	ccttcaatatcatcttctgtcgCAAGACGGACAAAAACGAGCAC
	02458.3-3R	GTCCAAACGATGACCCATACGAA
	02458.3-5N	AGACCTGCGTATGTCGTGTGCTT
	02458.3-3N	GCGGATCCTTCTGCGTCGTTAT
Fg05334	05334.3-5F	GCTTTGGCTTGCTTGCTCAGTG
	05334.3-5R	gcacaggtacacttgtttagagAAGATATCGCCCAAGACGGTCATA
	05334.3-3F	ccttcaatatcatcttctgtcgGGAGATGAAATCTGCCGAAGAGA
	05334.3-3R	CCTCATAGGTGCGGGGTTGATAG
	05334.3-5N	TCTTTGGCTGCTTCATCATCGTC
	05334.3-3N	GACTTTCGCTCCGATGTTGTCAC

	RT65-R	GGTGCACCATTTTCCGAGAGC
Fg01972 FGSG_01972.3	RT67-F	GCATTCGCACCCAACTTTCTCTA
	RT67-R	GTCGGATAGCTTCTCGCCTGTTT
Fg08187 FGSG_08187.3	RT68-F	TTGGAATGTCTATGGCTGCTGTTG
	RT68-R	ATCGAGCAATCGCATATTTGTAAGC
CYP51B FGSG_01000.3	RT69-F	CTTTACTGCTTCCCATGCTCTCCA
	RT69-R	CGCGGCGCTCCTTGATAGT
Fg08186 FGSG_08186.3	RT70-F	GGGCGCCTTCAATTGCTACAC
	RT70-R	ATCTGGTGCATGATCCTCTTGACA
Fg08183 FGSG_08183.3	RT72-F	TTATGGAGAACGGGTGGCAAAA
	RT72-R	ATTTTGGCGTTGCGTCTCCTTA
CYP51C FGSG_07977.3	RT73-F	ATCGCTCCATCAAACACCATTCA
	RT73-R	TCGAATGCATAGCACTGGAGCC
Fg08005 FGSG_08005.3	RT74-F	AAATATTTCGACGGTGACGCAGAC
	RT74-R	GGTGGCTGATGCGAGGAAATACT
Fg10629 FGSG_10629.3	RT75-F	ATTTGATGACGCCGACTTTTCTGT
	RT75-R	TTGAGTGTCAATCCCAAAAGAGGC
Fg10695 FGSG_10695.3	RT76-F	TGCGAAAGCGACATATCAAAAACA
	RT76-R	GGTGAGACATGTAGCGGTGGTTTC
Fg10910 FGSG_10910.3	RT77-F	CAATGCAGAGCTTTTGGAGGACA
	RT77-R	GGAAAGGGGCCCAGACAGG
Fg11389 FGSG_11389.3	RT78-F	TTTGATGGGTTTGCCGATTTTG
	RT78-R	AGCCAAGCCGACACAAAAACAT
Fg11424 FGSG_11424.3	RT79-F	GATGAACCGGTATGCCAATGACA
	RT79-R	AACTGCGACGTAAGCATTGAGAGC
Fg11282 FGSG_11282.3	RT80-F	TTCTCAAGCCCTTCCCCAACTT
	RT80-R	TGCAGGTAGTCGTTCCGCTCAG
Fg11303 FGSG_11303.3	RT81-F	AATCAGCGGTTTCTTGCGTCAC
	RT81-R	CGACTGGTGATCTGGATGCTGTTA
Fg11038 FGSG_11038.3	RT82-F	GCTACTGTTTCGGTGAGGCTTTTG
	RT82-R	GTACGGACAAGCATCGCAGCAT

Fg02672	02672.3-5F	GCCGATTATGCTTGCTCTCCTTAT
	02672.3-5R	gcacaggtacacttgtttagagGCCTCCATCATGCATGTCTTGT
	02672.3-3F	ccttcaatatcatcttctgtcgGGGGAGTGTTGAAGGAATGGA
	02672.3-3R	GCTACTCTCCTGGCCCCTGTC
	02672.3-5N	CTTTGCGGTCATTCCTTGCCT
	02672.3-3N	CCCAATACCCTCATCCCACCTTA
Fg02668	02668.3-5F	TTCATCGCGATTGATTCTGGTTAG
	02668.3-5R	gcacaggtacacttgtttagagGGCTTCGAACACCAGCAAAATC
	02668.3-3F	ccttcaatatcatcttctgtcgCCCATCCAGGAATACTACTCGTTG
	02668.3-3R	TTACCAGCTGCACCAAGCCTATC
	02668.3-5N	TCTTGGTACATACGCGGCAGAAT
	02668.3-3N	CAAAGGTAGCGCGAAAGTATCCG
Fg02929	02929.3-5F	AATGATGGGCGTGGTTGACTTACT
	02929.3-5R	gcacaggtacacttgtttagagACAAGGGCGAAACCTCTGGAAT
	02929.3-3F	ccttcaatatcatcttctgtcgCAATGACCTTCTCCCATGTAACCA
	02929.3-3R	TCCATTGGCGACCGAAGTTTT
	02929.3-5N	GATAGGACAGCTTTCGGGACCAC
	02929.3-3N	CCAACAGCGCCTGCCTTAGAC
Fg02872	02872.3-5F	CTGATGTTCCCTCAGATGGTTCCT
	02872.3-5R	gcacaggtacacttgtttagagTCGCTATAGCCGCTGTAGTCCAC
	02872.3-3F	ccttcaatatcatcttctgtcgGGTGCGCAGATCAAGAGTAGACAA
	02872.3-3R	TCGTCAGCCATGGCAAGAAACTA
	02872.3-5N	CACAGTACCGAGGCGACGAGAC
	02872.3-3N	CAGGGTTCTCTCCAGCTTTTGACA
Fg03548	03548.3-5F	AGGCGTGTATGAACGAGCGACA
	03548.3-5R	gcacaggtacacttgtttagagGCAAAATGGGAGGCAACAATACT
	03548.3-3F	ccttcaatatcatcttctgtcgCAGGATGGACCGATTGAGTATGAC
	03548.3-3R	CCTACTGACACGGCCACTGAGAA
	03548.3-5N	GAGTGGCTTCAGTTTGGGCTTCT
	03548.3-3N	GCACCTTCGAGCTGCATAAGTGT
TRI11	03540-5F	GGTCCCGCCTGGCCTCTTTA

Fg11108 FGSG_11108.3	RT83-F	CCACTAACCACCGGGAGACTACG
	RT83-R	ACCCTTCGCGTTGTGAGCAGT
Fg10960 FGSG_10960.3	RT86-F	GGAACTCGATTGATCACCCACCTA
	RT86-R	CGCAAAAACAGACTCGAAAATGG
Fg10991 FGSG_10991.3	RT87-F	GGATAGAGTGTTGGCTGCGATGAT
	RT87-R	CTTGCTAGAAGGTTGCGGTCATTT
Fg11002 FGSG_11002.3	RT88-F	GCTGCTGCCCGTAAGGAACTC
	RT88-R	TAAATCCGTGGTCGTGTGAATGG
Fg11024 FGSG_11024.3	RT89-F	GAAGTCATGGCCGAAATCACAATC
	RT89-R	AGCATGGTTTCGACGGAAGTTTT
Fg11465 FGSG_11465.3	RT90-F	CAAATGGTGGCGATGTTTAGCAG
	RT90-R	TGGAGAAAGCTGGGTAGCATCACT
Fg11498 FGSG_11498.3	RT91-F	GAGCTTTTTTTCCATTCACCGTCA
	RT91-R	TTCCTGGCTGAGTCCATTTTTGAT
Fg11536 FGSG_11536.3	RT92-F	GTCAACATCCATCACAGCCTCCTT
	RT92-R	TCTCTTGACCTCAGGCTGGCTCT
Fg10451 FGSG_10451.3	RT93-F	GGCCAAGAACGGTAAGCGAGA
	RT93-R	CTGTCGGGTGTGTCTCTCACTTCA
Fg10461 FGSG_10461.3	RT94-F	GTTAAGGAGCTGACCCCCATTTTC
	RT94-R	TCTCGTTGCTGGGGTTGTGC
Fg09351 FGSG_09351.3	RT95-F	CAAGCGCAAGCCTGTGGACT
	RT95-R	AGCTCAGCGACCATCTTTTCACAA
Fg09195 FGSG_09195.3	RT98-F	CTGCCAGCCGAAACAAGGAGT
	RT98-R	GTCGACGCGCTGCTTCAAGA
Fg09916 FGSG_09916.3	RT100-F	AGCGAAGGCCAATCTGAAAATG
	RT100-R	GAATCTGCTGCAAGGCGTGTC
Fg09671 FGSG_09671.3	RT101-F	CAGCTGGTGCGGGTTTTGTCT
	RT101-R	CTGCATCATGGTGGTGAAGGTCT
Fg11655 FGSG_11655.3	RT103-F	ACCGGTCAGGGGTTCACTAAAAAT
	RT103-R	TTCAAGGCCCGACCCAACTT
Fg12534 FGSG_12534.3	RT104-F	TTTGACTTTGCGCCATTCTCG

	03540-5R	gcacaggtacacttgtttagagTCACGTCTTAGGTTCAACGCAGTA
	03540-3F	ccttcaatatcatcttctgtcgCCTCAAAGCCAGCGATGATAGAT
	03540-3R	TCGGCGCAGAGACTACGGATAC
	03540-5N	AGGCATATCCGCTGGCAAATACT
	03540-3N	TCCATCTATCCAACCAATCGTGCT
TRI4	03535-5F	GTGAAGGTGGGAAGGGCGATAA
	03535-5R	gcacaggtacacttgtttagagTTGTTGAGTAGAAGCCGCCAGATA
	03535-3F	ccttcaatatcatcttctgtcgCACTGTATCGGCATCGAATCCTG
	03535-3R	GAGTTCGACGACAAGTGCACAGAAT
	03535-5N	GTCAAAGTCGAAGCAAACAAGTGGT
	03535-3N	AGGTATGAAATTTCGACCAGGGCT
Fg03542	03542.3-5F	ATGGAACCGAGATGGCTCAAAATA
	03542.3-5R	gcacaggtacacttgtttagagGAGTCTTTGTGCTTACCTCTCCTT
	03542.3-3F	ccttcaatatcatcttctgtcgGTTGTTTTGGCAAAATACTGGGC
	03542.3-3R	CCCAGGAGACACCAAGGAGAAAG
	03542.3-5N	GCCACTGCGTGTCTCAATAAACC
	03542.3-3N	AATGGTCGTCGCCCAAATCTATC
Fg03498	03498.3-5F	TGGACAAGGAACCCATCACACG
	03498.3-5R	gcacaggtacacttgtttagagCACGAAGCATGATGATGACAACA
	03498.3-3F	ccttcaatatcatcttctgtcgGCATCTGGGTAGAGTGATGACAGG
	03498.3-3R	AAGTCTCCGACACTCGTCAAAAGG
	03498.3-5N	GGCAGAGATCTACGGGTTCCATT
	03498.3-3N	GTTTCTGTCCAGGCGGCTAATG
Fg06068	06068.3-5F	TTATGGCGTTGATGTGTGGCTTT
	06068.3-5R	gcacaggtacacttgtttagagACCCCAGGTACACCCACAGATG
	06068.3-3F	ccttcaatatcatcttctgtcgGGACAAACAAAGCATATGACGGA
	06068.3-3R	TGGCATTCTGGTTGTTCGCTGTA
	06068.3-5N	AACGAGCATCACATGGACTTTTGA
	06068.3-3N	GTTGTGATGGTTGACCAAAAACGC
Fg01786	01786.3-5F	GCGAGCAGCCCTACACCTTTACT
	01786.3-5R	gcacaggtacacttgtttagagGCAAGGTGAAGTTCTGCGAAAGTC

	RT104-R	TTTGCCCAGGAAAGTGCTCTTG
Fg12568 FGSG_12568.3	RT105-F	GTACGTTGCCGGTTTGGTTGAA
	RT105-R	ACAACGCGGTCGATCTCTTCC
Fg08809 FGSG_08809.3	RT106-F	TCTTGTCAACGCTTTACCCGAGTC
	RT106-R	GTAGATCGGGCCCTGGTAGACAG
Fg13176 FGSG_13176.3	RT107-F	GTGCTTAAACCGGCGACTGCT
	RT107-R	ATGAGGTCGCGATGAATCTTGG
Fg12737 FGSG_12737.3	RT108-F	CACCAAGTTGCCGCTGACG
	RT108-R	GCTCGCTGCTCCATTCTGTTG
Fg12599 FGSG_12599.3	RT109-F	TCGGAATTCTTGTTTGGCAGTTG
	RT109-R	CAACCAATTTTTCGGTCTGCCTC
Fg13445 FGSG_13445.3	RT113-F	TGGCTATGGACGCGTTGACTG
	RT113-R	TTGGCGTAGGGCTTGAGTTGG
Fg13459 FGSG_13459.3	RT114-F	AGGAGCGTTCAGCCTCACAAAC
	RT114-R	AGCTTTTGCGCGAAGTTGACC
Fg14007 FGSG_14007.3	RT116-F	GGTTGGAGCCCGATAAGGATGT
	RT116-R	ACATCAAGCCCCAGTCCAACG
Fg13739 FGSG_13739.3	RT117-F	TTCGTCACCACTGGAGCAAAGATT
	RT117-R	CCGTTCTTGTGCAATAGCCAGTC
Fg13796 FGSG_13796.3	RT118-F	AAGGTCTCGGCTCCAAAGGTTC
	RT118-R	CCTCGGCGAGATGAAGAACTTG
Fg13797 FGSG_13797.3	RT119-F	ACCCTCACTTTGGATCACGACTGT
	RT119-R	GCCTCGAAAAGAACGGGGTC

	01786.3-3F	ccttcaatatcatcttctgtcgCGATCCCGATTACGAGATTTTGTT
	01786.3-3R	TCACGACGGGCTGAAAAAGAAAC
	01786.3-5N	CAAGGATTGGGCTGGATGCTC
	01786.3-3N	TGGATTGTTTGTGTCCCCGTGTA
Fg01767	01767.3-5F	TGACCACCGGCTACTCCACTTCTA
	01767.3-5R	gcacaggtacacttgtttagagCAAAGGCCAACAGAACCACCAC
	01767.3-3F	ccttcaatatcatcttctgtcgTCTCTCGGTTGAGGTTCACATCG
	01767.3-3R	CAAAAGAAGCGCCTCGGTGTAG
	01767.3-5N	CCTACCGTGCCCACCACTTACA
	01767.3-3N	TCATCAGCTTCATAGGCCATCTCA
Fg01745	01745.3-5F	ATGTTGCCCATGGTACTGGTTAGC
	01745.3-5R	gcacaggtacacttgtttagagTGCTGGGCGAGGACACTTAGA
	01745.3-3F	ccttcaatatcatcttctgtcgCGCTGCTCTTGATGATAGTTCCA
	01745.3-3R	CGGCCCTTCAAACAACTCACCT
	01745.3-5N	CGCAACCAGTGCACAAGTTTACA
	01745.3-3N	TTCGGGCACCAACACCTCTTC
Fg01740	01740.3-5F	CGTCTGCCTGTTTTGTGGTTGACT
	01740.3-5R	gcacaggtacacttgtttagagTCGGTAGTTCCATGGTGTGATAGG
	01740.3-3F	ccttcaatatcatcttctgtcgGCACCTTGAGAGGATGATGGAAC
	01740.3-3R	AATTGTTCCACAAGTTCCACGAGC
	01740.3-5N	TTCTTAGGCGCCTTTGTTATGACG
	01740.3-3N	TCGCGACGAGCTCAAGGACA
Fg01739	01739.3-5F	TGTCTTTGGGGCGGAGAATCAT
	01739.3-5R	gcacaggtacacttgtttagagGATGTTGAGGCTGTCCATCTTGAG
	01739.3-3F	ccttcaatatcatcttctgtcgAAGGGTGTTCGCGTCACTGTC
	01739.3-3R	CTGGCGATGGCTTGAGTATCCT
	01739.3-5N	GCTCACAAGGCCGGACATGGTAT
	01739.3-3N	GTCAAGGGTAATGCCGTGAATCG
Fg01722	01722.3-5F	TCACATAGGGTCGGCAGACTTTTG
	01722.3-5R	gcacaggtacacttgtttagagCCCAGAGACTATTTCGGCTGTGA
	01722.3-3F	ccttcaatatcatcttctgtcgCGGTAACCAAGGGGGACAAGT
	01722.3-3R	GGCTTCCAGTAAACCAACGTTTAT

	01722.3-5N	CGGTTGGTTAAAAAGCAGTGTGGT
	01722.3-3N	CGTTTTCCGCCAGAGATGAGTTC
Fg05113	05113.3-5F	TTGAGAGAACCCAGGACCGTAATC
	05113.3-5R	gcacaggtacacttgtttagagCCATCTTGAGTAAACCTCAGCAGC
	05113.3-3F	ccttcaatatcatcttctgtcgCCCAGAGCACTTCGATTGTGTCA
	05113.3-3R	TTGTGCGCCATCATTCCATTG
	05113.3-5N	TGAGTCGCTATTGTGGATGGTGC
	05113.3-3N	CCTCGAGCGGCTGTTCATAGAT
TRI1	00071-5F	TGATGACAGGTGAAAGAGGCTGGT
	00071-5R	gcacaggtacacttgtttagagGGTCTGTCAAGCCTTGGAAGAGAT
	00071-3F	ccttcaatatcatcttctgtcgGCGATTGGAATTGGTACAGGATG
	00071-3R	ATGTATGAGCAGCGAGGTTTAGCC
	00071-5N	ATAGATCATGGGGGAAACAGTAGGC
	00071-3N	CGACTTTACCGTTGGCTCTTTTCA
Fg01583	01583.3-5F	CCTTGCCAAGACCATTGAACATC
	01583.3-5R	gcacaggtacacttgtttagagGCTACCAAGAAGAATATGAGGGTT
	01583.3-3F	ccttcaatatcatcttctgtcgGATATCACGTTCCATCCCTTGAGA
	01583.3-3R	CGTGCCAAGAGAACGTATGAATGA
	01583.3-5N	TGACAATGACTCGGCACTGGTGT
	01583.3-3N	ACAGGATTCTTGCATCGGACCAT
Fg01048	01048.3-5F	GACAACGCAGGTCAACCTCATCA
	01048.3-5R	gcacaggtacacttgtttagagGCGTCTTAACTAACCCAGACCAAGG
	01048.3-3F	ccttcaatatcatcttctgtcgTTGGGCGTTGGTATTTGGTTGA
	01048.3-3R	CTTGGACAGACGTGCCAGATTAGAG
	01048.3-5N	CCGGTAGCTCTAGTAGGGCATTCAT
	01048.3-3N	CTGATACTGCTGTGGTCGTTGTGG
Fg02419	02419.3-5F	GGGTGGGTGAGTGAGTAGCATACAA
	02419.3-5R	gcacaggtacacttgtttagagTAATTGGTTGGATGTGGACCTGAAG
	02419.3-3F	ccttcaatatcatcttctgtcgTTCCATCTGTGACCCTACAAAGCC
	02419.3-3R	GGAACAAGCCAATTGTCCGTAGTG
	02419.3-5N	CTACTTGCAGCGTCGTCCTATT
	02419.3-3N	TGCACCAACCAACAAATCCCATA

Fg02371	02371.3-5F	GACAATAAGGCACCATACTGAAACCC
	02371.3-5R	gcacaggtacacttgtttagagCGACGTAGATTGCAGATATGACCCA
	02371.3-3F	ccttcaatatcatcttctgtcgTGCATCAGGATTTGGTACGGAGTC
	02371.3-3R	CACCAGCTCCCCTCATCATCCT
	02371.3-5N	GGGGTCGGAATGTGTGGATTTC
	02371.3-3N	CGACATTTCACATCCACAGTCTTCC
Fg02367	02367.3-5F	TGCTCGCATGCTCCATTTGAC
	02367.3-5R	gcacaggtacacttgtttagagGTATCGTGATGGAAAACTGTTCGGA
	02367.3-3F	ccttcaatatcatcttctgtcgCGGTTGGAGGTTAGAATTTCGCTAC
	02367.3-3R	AAAGGAAGACATCTCGAAATCTGGTG
	02367.3-5N	GGCATCGCTAACCTTTTTTGTCG
	02367.3-3N	AGAGATCTGGAGATTGTTCCTGCGTA
Fg02366	02366.3-5F	TCCCAGAAACACCACCGCATAG
	02366.3-5R	gcacaggtacacttgtttagagGCAAAGCTCAAAATAAGAGTCCAAAGG
	02366.3-3F	ccttcaatatcatcttctgtcgGCAGGCGCGATACTTTTTACACA
	02366.3-3R	GCCTGTCTTCCGAAGTTTGATGTTT
	02366.3-5N	TAGCTTCTGTCCCAGTCCGTGTTT
	02366.3-3N	GGAGCTGGTTGACAAGTCGGATAA
Fg02344	02344.3-5F	CGCACCGCAGTGTTCAAAATG
	02344.3-5R	gcacaggtacacttgtttagagAGAACAAAGGACTCAGAGCGACCA
	02344.3-3F	ccttcaatatcatcttctgtcgGACATTTCAGGCTCAACGTGCTCTA
	02344.3-3R	GCCGTGGGGAATGCTGTTTAC
	02344.3-5N	CTGGAAGGTGGGACACTAACATCAA
	02344.3-3N	CTTAGCTAGAACGGGCCAAGTCAATA
Fg03796	03796.3-5F	GACATGCATCCAGCCACAGTAATCT
	03796.3-5R	gcacaggtacacttgtttagagAAACACCCAAACTTCTGGCGTAAAT
	03796.3-3F	ccttcaatatcatcttctgtcgGAGCCGCAGCATGGTATCAAAGT
	03796.3-3R	GCTTTACCCCAGCCACGGAATA
	03796.3-5N	CCTGGCAATAAAGATTCCTACTGGC
	03796.3-3N	GGAAAGTTCCTCTGGGCTGGTG
Fg03741	03741.3-5F	CTGAGCTTGTTGCTGCCATCTTG
	03741.3-5R	gcacaggtacacttgtttagagTCTGGCTCGGCTAGAAGCTTTACTG

	03741.3-3F	ccttcaatatcatcttctgtcgCGGTGGATAAACAATTAGCAATGGA
	03741.3-3R	TAGCAGCCCAGGTCTCGTAGTCAG
	03741.3-5N	CAGGTGAATGCTTCGACTTCTTTCTC
	03741.3-3N	GCTCTTGTCGACGAACTGCTGG
Fg00863	00863.3-5F	CGACGGTCAGCATCTTCTCTTGTAA
	00863.3-5R	gcacaggtacacttgtttagagTCTGGCTCGGCTAGAAGCTTTACTG
	00863.3-3F	ccttcaatatcatcttctgtcgCGTAGCACTTCGGAGGCTTTGAT
	00863.3-3R	GAGGAGGCGAAGCAAAGACAATC
	00863.3-5N	TCTCATCAGAAAGGCCATCATTCC
	00863.3-3N	GCTGGGGTCTCCTCTGTCTTGATT
Fg00012	00012.3-5F	AAGGCAACTCCAACGGCGATA
	00012.3-5R	gcacaggtacacttgtttagagTTTGAGACGAGGAGGGGGATTG
	00012.3-3F	ccttcaatatcatcttctgtcgGCAGGGTACGCCTTTTAAAGACTACA
	00012.3-3R	CTGAGAAGCAGTCAAGGAGAGAATGG
	00012.3-5N	GCCTATTAAATACCATTGCAAGCGTT
	00012.3-3N	ATGATAATGAACGGTCTACTCGCCC
CLM2	00007.3-5F	GTTGCAGATGCCAGCACACTTG
	00007.3-5R	gcacaggtacacttgtttagagTGATAGGCAAGGGTTTGGGTCC
	00007.3-3F	ccttcaatatcatcttctgtcgCTTGCTAAGAGAAAATGGGGGTTGA
	00007.3-3R	TGTCATTGCACCTGATCTTGAAACA
	00007.3-5N	AGATGGGCTGGAAATGACCGTAA
	00007.3-3N	GGCACAGGTAGATACCAGCGTCAT
Fg00127	00127.3-5F	TCCGAGATCCGAATTAATACCACATC
	00127.3-5R	gcacaggtacacttgtttagagTCCACGTGATCACATTAACCATTCTC
	00127.3-3F	ccttcaatatcatcttctgtcgCTCGCGCTCTGGGTTCTGAAG
	00127.3-3R	CAAATTTGGATTGCCATCTGTAGACA
	00127.3-5N	TACAATGCAACAGCGGTGGTAGAG
	00127.3-3N	TTAGCGATACCCAACCTCATCCTG
Fg08412	08412.3-5F	TGATGTGATTGCTGCCAATGTAGG
	08412.3-5R	gcacaggtacacttgtttagagGATGGTTGTGTTAAGAGTGGAAGGGA
	08412.3-3F	ccttcaatatcatcttctgtcgCTTGGGCTTCAACGACAAACTGAT
	08412.3-3R	TGAATCCTAGGCAAGATACTCCCCA

	08412.3-5N	ATATGGGATATCGCGCTCTTGGTAA
	08412.3-3N	CTCCAAGATCCACAAGAGGCTATGTT
Fg08377	08377.3-5F	CAGCCGAGCAGATCATAGACAGGT
	08377.3-5R	gcacaggtacacttgtttagagAGAAGTTGTGTCGGTTTCACGGAC
	08377.3-3F	ccttcaatatcatcttctgtcgTTGACCCCTGGTATCGCTATGCT
	08377.3-3R	CAAGGCATGACGGAGACACAGAA
	08377.3-5N	GCGAGTCAATTCCGGTGTATCTGT
	08377.3-3N	TCGCCAACCAACATAAAACAGACC
Fg08320	08320.3-5F	ACGAAGGAGCCGCAGGTATTTT
	08320.3-5R	gcacaggtacacttgtttagagGGAAGGAAGGAGGCTTGTTGGAG
	08320.3-3F	ccttcaatatcatcttctgtcgGCTGCCACATATCATGATTTCAAGAA
	08320.3-3R	GGTACTCGGAAAAGCGACAAACAG
	08320.3-5N	ATTCAAACCGTCGAGCTTAGACCC
	08320.3-3N	TGAGAGTCCTTGGGGCTTTTTGTA
Fg08207	08207.3-5F	GATTCAAGAAACCATGCCTCCGAT
	08207.3-5R	gcacaggtacacttgtttagagCAGTGTGTGATTGGGTCGTGTTTG
	08207.3-3F	ccttcaatatcatcttctgtcgCTGAGATTGGCTTTGTTGGAATGAA
	08207.3-3R	GCTCTGGTTTTAGCGTCGGGTAG
	08207.3-5N	GGAAATGAGCGAGGTCTTGGAGA
	08207.3-3N	GATATTGCGTCTTACCCCCTGAGAG
Fg01972	01972.3-5F	CGATGAACTTTGGGAGATGTGGAC
	01972.3-5R	gcacaggtacacttgtttagagAGGTGAAGATGCAGGATGAAGGAGT
	01972.3-3F	ccttcaatatcatcttctgtcgATCTGTTCAGGAATCCTCCGTTGAT
	01972.3-3R	ACACACCAACCAAGGCTTTGTATTTC
	01972.3-5N	GAGGGAGAAACTGTCGCAAATCAA
	01972.3-3N	TGGGAGCTAACCAACCTTCCAGTATA
Fg08187	08187.3-5F	GCAGTGCTCTCGTTGAAGTGGTT
	08187.3-5R	gcacaggtacacttgtttagagAGTCCCCCACACCTAGCGTAGTG
	08187.3-3F	ccttcaatatcatcttctgtcgGGAGGTCAAGCGCAGGTCATAG
	08187.3-3R	GATGACGTCCAGGTGCCAAAGT
	08187.3-5N	TGCGCTACCAGACACGGAAAGT
	08187.3-3N	ACTTCGACCTGGCTTTCCTGTGTA

CYP51B	01000.3-5F	CGGTATCGTAGCCTCGACTCCAT
	01000.3-5R	gcacaggtacacttgtttagagGCCAGTTCTTGAAGGAGACCCAT
	01000.3-3F	ccttcaatatcatcttctgtcgGGGAGCGACGCCAGTAAACTTA
	01000.3-3R	ACAGGCACACAAAACACTCCAGC
	01000.3-5N	ATGGGCCATCAACACCTCAAAA
	01000.3-3N	CTTAGATAGCGGGCCAGGGAGAT
Fg08186	08186.3-5F	AATGGTTCCTCCCCGCTTGTAA
	08186.3-5R	gcacaggtacacttgtttagagTTGAGGGCGATGATATTGAAGTCA
	08186.3-3F	ccttcaatatcatcttctgtcgTTTGCGTTGGTGAAAGGTGAGTC
	08186.3-3R	GGCTGCTGGCACATCACTTACAT
	08186.3-5N	CCTGAGGTGTACGAGAACCCAGAC
	08186.3-3N	CCCAGACCATTTGCGTCTCAGTAT
Fg08183	08183.3-5F	CCTGCCACTCATTCTTCAACACC
	08183.3-5R	gcacaggtacacttgtttagagATAACGCCGATGAGGATGATAGGA
	08183.3-3F	ccttcaatatcatcttctgtcgCGCTTCATCAGACGCTAAGGATTT
	08183.3-3R	CGGCTAAAGCTCCCTCAACACTG
	08183.3-5N	GTGCTACTCCGTTGTGACTCCCTC
	08183.3-3N	GGACTTGGCCCAGGAGTAAATGA
CYP51C	07977.3-5F	CCCAGGCAATGGTGACAGGCTA
	07977.3-5R	gcacaggtacacttgtttagagCCAGTTAAGGAACGGCATCTTTGT
	07977.3-3F	ccttcaatatcatcttctgtcgTAATTTCTTTGTCCGGGTTCGTTT
	07977.3-3R	GTACTGCAACCAACTGGTCCGAG
	07977.3-5N	CAGAGTAAGCATAAACGCCGCAC
	07977.3-3N	GACCATGGCCAACACAATACGAG
Fg08005	08005.3-5F	ATGGATGCGCCCAACAGAAAT
	08005.3-5R	gcacaggtacacttgtttagagCATGATGGGATTGTGCTCGGA
	08005.3-3F	ccttcaatatcatcttctgtcgCCTCCAGTTCCGCTCAACACTC
	08005.3-3R	ACAGTCATCAGCATCGCAAGACAT
	08005.3-5N	ATTCGGGCTGTATCTATCTCACGG
	08005.3-3N	CCAAACCCTGGTTTTCGGACTAT
Fg10629	10629.3-5F	AAATCGAGCAGGACGCTAAGAACA
	10629.3-5R	gcacaggtacacttgtttagagCGCCATATTGTGCCTGTATTCGTA

	10629.3-3F	ccttcaatatcatcttctgtcgCACCCGGATCGAGTCTTTATCAA
	10629.3-3R	TAGAGGGGGTTGCAGCGTGAA
	10629.3-5N	ATTCAGTCCACTCACCGAGAACCA
	10629.3-3N	GACTCATTCGCCAACCCAACATA
Fg10695	10695.3-5F	GATGTCCATGAAGACGGGTTGTG
	10695.3-5R	gcacaggtacacttgtttagagACCAAAAGGCCACCAAATACAATC
	10695.3-3F	ccttcaatatcatcttctgtcgGTGGAAAAAGCCTCGTTTGATGG
	10695.3-3R	CTGTTCTGGACATGGCGGTGAG
	10695.3-5N	GGGCAGAATGCGTTCTATGACAG
	10695.3-3N	TATCATGTCGTCAGGGTTGGGCT
Fg10910	10910.3-5F	TAGTCGGTAGAGCGCTGGATTGTA
	10910.3-5R	gcacaggtacacttgtttagagGGCTATCCATAATGGGCGACAGTA
	10910.3-3F	ccttcaatatcatcttctgtcgGCAGCCATGGAGCTCTCTTAGATT
	10910.3-3R	CTCTGGAGAAAAGCATGGTGAAGC
	10910.3-5N	AAACAAAACGAGGCGACAAAACAA
	10910.3-3N	TAGACGTTCGGTTTTCAACAGCAA
Fg11389	11389.3-5F	GAACGGGGCGTCTTGATTACCT
	11389.3-5R	gcacaggtacacttgtttagagCACCCAGCGCCAGAAAGAATAC
	11389.3-3F	ccttcaatatcatcttctgtcgTTGATCCTGTAACAAAGCAGTGGC
	11389.3-3R	ACTGTTGTTGTCGGGGAAGAAAAA
	11389.3-5N	AGAAACTTCCGAAGGTTCCGAGG
	11389.3-3N	CGTTTTCACTCGCGGTCCTATG
Fg11424	11424.3-5F	ATTCATCATGGTTTCTGGTCTCGG
	11424.3-5R	gcacaggtacacttgtttagagTCTGCCTCCCTTTTAACGCTACAA
	11424.3-3F	ccttcaatatcatcttctgtcgATTCCCGTAAGTCAGCCAACCAC
	11424.3-3R	ACCTGAAAATGCACATCCTCGTCT
	11424.3-5N	AGGCGGACGGTATCTTAGGCAA
	11424.3-3N	TGAGTTGAGGATGAAAGGGAAACG
Fg11282	11282.3-5F	CGCATGCGCTGTCTCAGTAAGTAG
	11282.3-5R	gcacaggtacacttgtttagagACCAAATCTGTAGCCGCCTGTATC
	11282.3-3F	ccttcaatatcatcttctgtcgGTCGAACGATTTGTGCGGAGA
	11282.3-3R	GACGTCGATACCCAGTGCTTGTG

	11282.3-5N	TGCTTCCATATGCTACCGTCCCT
	11282.3-3N	CAGGCTGACTCCAAGAGCAATGTT
Fg11303	11303.3-5F	AGAGGAGGAGTTGGATGGTGAGGT
	11303.3-5R	gcacaggtacacttgtttagagCATGTTGACGGTGGTTGGTATCA
	11303.3-3F	ccttcaatatcatcttctgtcgTATGACAAAGGAAGGCGGCAATA
	11303.3-3R	CAGCGTTGCCATTACCAGGAGA
	11303.3-5N	TCAAGCCCTCGAGAGCTATGATGT
	11303.3-3N	ATTTGCAACTGGAAGCCCTGTATG
Fg11038	11038.3-5F	ATCTGCATGCTCCAAGAGACGACT
	11038.3-5R	gcacaggtacacttgtttagagTCGCACCCTGAACAGAGTCGTA
	11038.3-3F	ccttcaatatcatcttctgtcgAGGGAAGCACCGGAGTTAGAGTG
	11038.3-3R	GCCGTTATACCGTCATTGGACTCT
	11038.3-5N	ATGTATGCGCTGAAGTGTCTCCCT
	11038.3-3N	ACCGCCCATTGTCCTGAACTG
Fg11108	11108.3-5F	ATCGCTGCAGTCGTTTCTTTTGA
	11108.3-5R	gcacaggtacacttgtttagagTGGCTTTGGGGAAATAACACGAT
	11108.3-3F	ccttcaatatcatcttctgtcgTGACATTATGCCCTGGCAGATTC
	11108.3-3R	CACAATCGAACCAGTCAGCCACTA
	11108.3-5N	GTCGCATACGTCGCAGAGAACAC
	11108.3-3N	TGACCTGCAACCAACAGAACATTT
Fg10960	10960.3-5F	GGTCATCAAGAATGAACGGGGTG
	10960.3-5R	gcacaggtacacttgtttagagATTTTTCCTTGCCATATCCATTCG
	10960.3-3F	ccttcaatatcatcttctgtcgGTCCGTCATATGAGTTTGTGGCAG
	10960.3-3R	CACAAAGCACAGCCCACTGAAAT
	10960.3-5N	GCGATCAGCAAGCTGAGGATGT
	10960.3-3N	AGATATCAACCCGACCCATTCCA
Fg10991	10991.3-5F	CATCGCACGACCATGGAAAAAG
	10991.3-5R	gcacaggtacacttgtttagagGCCAAAGCGACAATAGCTCTCATT
	10991.3-3F	ccttcaatatcatcttctgtcgCGCTGATAGCATTTGTCCTTTCTG
	10991.3-3R	TCGTGTCACCCAGCTTTGATGTC
	10991.3-5N	GGATGGGCACAGGTCAACACTC
	10991.3-3N	AACAGTGGCTCTCAACCATGACCT

Fg11002	11002.3-5F	TGAATTTGTCGACATCCCGCTTAT
	11002.3-5R	gcacaggtacacttgtttagagTGGGGAGGAAGGATCGTCAACT
	11002.3-3F	ccttcaatatcatcttctgtcgGAGCCCAGCAGGAAAAGAATAAGC
	11002.3-3R	GGGAGCAACTAGACACTTATCGCAAA
	11002.3-5N	GTTTCGATCATTTGGGCAAAGGTAG
	11002.3-3N	TGGCAAAGGATTATGATGGACTCG
Fg11024	11024.3-5F	CAACGATCGCTCATAACTACAAACCA
	11024.3-5R	gcacaggtacacttgtttagagCTCAGGCCGTAGACTGATAGTGGTG
	11024.3-3F	ccttcaatatcatcttctgtcgGTGTAGACCGAGCCCTTACCTTACCT
	11024.3-3R	TCTTGTAGGGAGGCATGGTATGGA
	11024.3-5N	AACATAGCAGGCAATATAGCGAAACC
	11024.3-3N	CACTTTCCTCTTGATAGGTTCTTTCGTC
Fg11465	11465.3-5F	CTCTCGCAGGCATACTCAGGTCTACT
	11465.3-5R	gcacaggtacacttgtttagagCCGACGCCACATGAGGTATGA
	11465.3-3F	ccttcaatatcatcttctgtcgAATGGCACCTCAATCAACGGAA
	11465.3-3R	TCACTGACTGATCGTCTGGTTCCTAA
	11465.3-5N	TTCCATCTTGTCCCAGCTGTTCTACT
	11465.3-3N	CCTCGTTACCCGTGGAATAGAGAAC
Fg11498	11498.3-5F	CCCTGAATACTATAAGCTTTCGCCCT
	11498.3-5R	gcacaggtacacttgtttagagCAAGTTTGAAAAAGGTGTGTTGGAAGA
	11498.3-3F	ccttcaatatcatcttctgtcgTCATGACCACTATTATTCACAGCCTTTG
	11498.3-3R	AAAAGCTATTGCGCACCCCCT
	11498.3-5N	GGTGGTGGTGCTCAAGATTTACTCC
	11498.3-3N	CTTTAGCTCCCAGCAAGCACGAT
Fg11536	11536.3-5F	CGACTCGTTTGGTCCCTTATTTCTC
	11536.3-5R	gcacaggtacacttgtttagagCTGGTCGACTACGCATGGCTAAAT
	11536.3-3F	ccttcaatatcatcttctgtcgCTAGCGAGGAGGACAGCGACTCT
	11536.3-3R	ACCGCTATGGGCATTTTATACGG
	11536.3-5N	TCGACTCGCATGGGTTTCCTC
	11536.3-3N	GTGGAACAAGGCATCGTTCAGTCT
Fg10451	10451.3-5F	AAGGAAAGAAAAGAGAGGGCGAAA
	10451.3-5R	gcacaggtacacttgtttagagTCATTTCAACGCCCGCATTCT

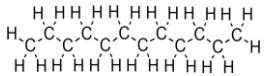
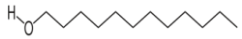
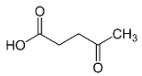
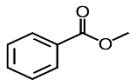
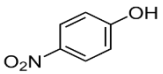
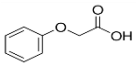
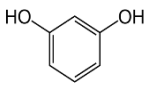
	10451.3-3F	ccttcaatatcatcttctgtcgTGGGATATCGTTGTGAGTAGATGGAAAT
	10451.3-3R	CCCTTTCACCAGTCCACATTTCG
	10451.3-5N	AGTGGTCGCGTGTTTGGTGTTT
	10451.3-3N	CAAAACCTTCCCACGACAGCAC
Fg10461	10461.3-5F	GAGTCATGGCAGAAACTAGAAAGGGA
	10461.3-5R	gcacaggtacacttgtttagagCGTGATGCGATGAAGCTGATTTC
	10461.3-3F	ccttcaatatcatcttctgtcgCCGGGCGGAGCTATATCTGACT
	10461.3-3R	TGCGCAAACATAAAGATCACTGGTT
	10461.3-5N	GCATTTCAAGCCCACATAAGATTCC
	10461.3-3N	GCATCTCAAACGAAATTCCACCAT
Fg09351	09351.3-5F	GAGACTGATGGAGAATGAGACCTTGC
	09351.3-5R	gcacaggtacacttgtttagagCAGCATGCCGTGGAACTGAAAT
	09351.3-3F	ccttcaatatcatcttctgtcgACTCCCTCTCTCCAACTTGCCACT
	09351.3-3R	GGCAACTATCGTCCCTATCCTAAACC
	09351.3-5N	GTGAGCTGAAGGAAAAAGAAAGTTGC
	09351.3-3N	TGCTGGTATGGGTAGATGCTTTGTG
Fg09195	09195.3-5F	AGCATCCCAGCAAGCCCACTA
	09195.3-5R	gcacaggtacacttgtttagagTGACAGGAAACCAGTCTTGCTTACG
	09195.3-3F	ccttcaatatcatcttctgtcgTTGAGTACAAGCTGGCCGCTAATAA
	09195.3-3R	GCAGAAAGATCAAGTTTTTGGAAGGTC
	09195.3-5N	TCTATAGAGCTTACAAACCAACCAACCC
	09195.3-3N	TGAGAGGGAGCCAGGTCTTCGTA
Fg09916	09916.3-5F	CGGGTGCGAGTTGAAGTTTTGA
	09916.3-5R	gcacaggtacacttgtttagagCGGACGATTCTCTGACATACCCTG
	09916.3-3F	ccttcaatatcatcttctgtcgCTGCGGGGACTATCGAAGGAAC
	09916.3-3R	GCGCCGTGACCAACTATCTCC
	09916.3-5N	AGAGGTTAGGATCGAAGGGGAGTTTA
	09916.3-3N	ACAATGCTGAAAGTTAGGGGGTCC
Fg09671	09671.3-5F	GCTTCATGTGGATGATACCCGAGT
	09671.3-5R	gcacaggtacacttgtttagagGGGTGGAACCACAAGCGATAAA
	09671.3-3F	ccttcaatatcatcttctgtcgGAGGAACAAACAGGAGGCGAAAC
	09671.3-3R	TCCTGCAGTGCGGCTATTGTTT

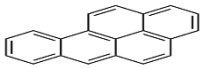
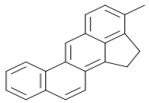
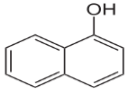
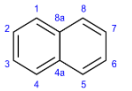
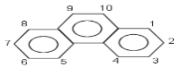
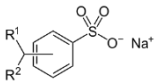
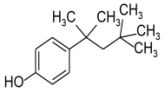
	09671.3-5N	GTGGCACAAGATATCGCAATCACA
	09671.3-3N	CCTCCAGCTCACACCCGATAAC
Fg11655	11655.3-5F	TCAGGCAACGGGTCGAAGATTA
	11655.3-5R	gcacaggtacacttgtttagagTTGGCGGTTGTGTAGGAGTTTTC
	11655.3-3F	ccttcaatatcatcttctgtcgCGTCCAGTCAGAGGCAGAGTAGG
	11655.3-3R	TCGTTGTTCCTGAGCCCTACCTC
	11655.3-5N	CTTGTGTGGCTGTGTTCGGATTT
	11655.3-3N	TCCATACCCTCTGAGAAGCAATCG
Fg12534	12534.3-5F	CGAGGAAGCTAATAACCGCAAAGA
	12534.3-5R	gcacaggtacacttgtttagagTTGAGCCAATTGTGAGAAACGAGA
	12534.3-3F	ccttcaatatcatcttctgtcgCCTTGTGTTGACCTATCTGCCGT
	12534.3-3R	TTACCCGGCACAGGAAGTCTACC
	12534.3-5N	GCACAGACGTCAACTGGTAAGCAT
	12534.3-3N	TGGAACTCGTGATCCCAAATGAC
Fg12568	12568.3-5F	ATGGAGCTACAGGACGGTGATGC
	12568.3-5R	gcacaggtacacttgtttagagCAAACAATTGCCCCAGAACAGAA
	12568.3-3F	ccttcaatatcatcttctgtcgATGTTTTCTTCCCCAGTTCGCTTA
	12568.3-3R	CCATGCACACACCAGCACTTGA
	12568.3-5N	CGCATCGGTGACACTATCCATTAC
	12568.3-3N	CTATCGTTTCGTGGCTCACAGAGA
Fg08809	08809.3-5F	TGGTGCTTCTTTCGGCATAGTCA
	08809.3-5R	gcacaggtacacttgtttagagTTACGGGCAGCAATGGTCTCTT
	08809.3-3F	ccttcaatatcatcttctgtcgACGCGAGTCCGAGATTGTGAG
	08809.3-3R	GATTCGGCTGGCGATAAAGGTT
	08809.3-5N	GGGTGGCTCATGGGTATCACG
	08809.3-3N	GCGGGAACAAGGGAAGGATAA
Fg13176	13176.3-5F	CCAGTCTGTCGCTCGCAAGTTC
	13176.3-5R	gcacaggtacacttgtttagagTTGAATCAACACAACGAGGCACA
	13176.3-3F	ccttcaatatcatcttctgtcgTCACCACTGGAGTCATCAGACCC
	13176.3-3R	CGTCCAAGGTACATTTCGTCCACA
	13176.3-5N	CCTGACTCTGCTTCTCGCACTTTC
	13176.3-3N	TCCATTCCAGATCCGTGAGCG

Fg12737	12737.3-5F	TAGTTTCCACACCACGCATCACTC
	12737.3-5R	gcacaggtacacttgtttagagGCGTGGGCCAGTATATTTTGTTTG
	12737.3-3F	ccttcaatatcatcttctgtcgAGGACATTGAAGGGAACATTGGTT
	12737.3-3R	AAGATAGTTGAGGATGGGCGGAG
	12737.3-5N	GCCGCCAGTTTCTATCACTTACGA
	12737.3-3N	GCTGCGACCGTGATAAGTCAACA
Fg12599	12599.3-5F	AATGATCTTGATGGCGAGGTGCTA
	12599.3-5R	gcacaggtacacttgtttagagCCGTTATCGTGTTTTACTGCTTCG
	12599.3-3F	ccttcaatatcatcttctgtcgTTCAAGAGGAAACCAAAAACCCG
	12599.3-3R	AAAGAAGGCTGTCCAACAAAACCC
	12599.3-5N	AAGGCCTCGACATCCTCAACAGT
	12599.3-3N	AATGATTCTCTCCACTCCAAGGCA
Fg13445	13445.3-5F	ACTTTTGGGAGACTATGCGAGACG
	13445.3-5R	gcacaggtacacttgtttagagCGTCTTGGTCCATAGTGGCTTTTG
	13445.3-3F	ccttcaatatcatcttctgtcgGGGATTGGCTCGACGATAAATGTA
	13445.3-3R	TTATCGTGGGAACGGCATTTATTG
	13445.3-5N	TGTTCAACGCGCCTTTTGGTATT
	13445.3-3N	GCTTTCCACCATGCCGTCAG
Fg13459	13459.3-5F	AAGGAGTGTAAGTGGGTCGTGTGG
	13459.3-5R	gcacaggtacacttgtttagagGCGACTATCTTGCAAATGGAGGTT
	13459.3-3F	ccttcaatatcatcttctgtcgTCGCCTGAGGTAGACATCTGGAAT
	13459.3-3R	CCATCCACAATGTCCGCAAAAG
	13459.3-5N	GTCGGATCACCCATCAAAGTCAAG
	13459.3-3N	ATCCATGCAGAGGAGACGGTGA
Fg14007	14007.3-5F	AAGGGGTTCGCCATTCGTGAC
	14007.3-5R	gcacaggtacacttgtttagagTTCTTCTGCTTCCTTTGTGCTCG
	14007.3-3F	ccttcaatatcatcttctgtcgGAGGGTCTCCCGATCATATAAACATT
	14007.3-3R	AACCGACAAATGTAACACCGCAT
	14007.3-5N	AGGCTCGAGAGAGAATATGGTGGC
	14007.3-3N	CGGCCCTATATCACGACCAAACT
Fg13739	13739.3-5F	CAGGGGGAGCTCTGGGATAAGA
	13739.3-5R	gcacaggtacacttgtttagagTGATGAGGTGTCGCATAGAAGGAA

	13739.3-3F	ccttcaatatcatcttctgtcgGGACAGCATGTTGCAGTCTTTGAA
	13739.3-3R	CCCAGTTTAGGCGCTGACTTCAA
	13739.3-5N	CAGCTGCTTTGGGAATTCTTTTCA
	13739.3-3N	GTGTGAGCCATTCCCTGTAGCCT
Fg13796	13796.3-5F	TCGTGAGAGGGCCAGACAATGTA
	13796.3-5R	gcacaggtacacttgtttagagTCCAGGGATTTTCAGGACTTTGAG
	13796.3-3F	ccttcaatatcatcttctgtcgGATGAGAGCCTCCGAAGCAAGTC
	13796.3-3R	GGGCACCGATCACGATAGTTTTC
	13796.3-5N	GCGACGATCTGTTGCCACTAAAG
	13796.3-3N	TGGAAACATGCTTGGTGGTCGTA
Fg13797	13797.3-5F	CACATACGTGCGGGATTTGAACTC
	13797.3-5R	gcacaggtacacttgtttagagTTCTTACATTGTCTGGCCCTCTCA
	13797.3-3F	ccttcaatatcatcttctgtcgAAGCGTGGCTGATGGTTGAAGTAT
	13797.3-3R	ACAAGCCATCGTTCGGGGTAA
	13797.3-5N	CGGTCTTAGTCAGACGGTCCAGTG
	13797.3-3N	GATACGTCTCGCGAAGACTGGAAC

Table 4. List of 15 xenobiotics.

Group	Compound name	Concentrantion (mM)	Structure	Description[a]
Aliphatics	n-Dodecane	176.12		Dodecane's production and use as a solvent, in organic synthesis, distillation chaser, in jet fuel research, standardized hydrocarbon, manufacture of paraffin products; rubber industry, paper processing industry, and as a component of gasoline may result in its release to the environment through various waste streams.
	1-Dodecanol	22.35		1-Dodecanol's production and use in detergents and soaps, in fragrances, and to a lesser extent in wetting, emulsifying, and foaming agents may result in its release to the environment through various waste streams.
	Levulinic acid	9.77		Compounds or agents that combine with an enzyme in such a manner as to prevent the normal substrate-enzyme combination and the catalytic reaction.
Aromatics	Methyl benzoate	14.69		Methyl benzoate's production and use as an additive in perfumes, disinfectants, soy sauce, and pesticides, an additive or preservative in cosmetics and personal care products, and as a solvent or dye carrier may result in its release to the environment through various waste streams.
	4-Nitrophenol	0.14		4-Nitrophenol is the most toxic of the mononitrophenols.
	Phenoxyacetic acid	0.53		Phenoxy acetic acid (an acid $C_8H_8O_3$) used as a fungicide and in plasters and pads to soften callused skin surfaces.
	Resorcinol	0.72		Resorcinol's production and use as chemical intermediate, in the production of sunscreen preparations for the skin, production of dyes, manufacture of special pharmaceuticals, manufacturing adhesives, explosives, tires and rubber products may result in its release to the environment through various waste streams.

Polyaromatics	Benzo(a)pyrene	0.32		There is no commercial production or known use for benzo(a)pyrene; it is released to the environment as a product of incomplete combustion. Benzo(a)pyrene is found in fossil fuels, crude oils, shale oils, and coal tars, and is emitted with gases and fly ash from active volcanoes.
	3-Methyl cholanthrene	0.15		3-Methylcholanthrene's production and use as an experimental cancer research chemical may result in its release to the environment through various waste streams.
	1-Naphthol	0.14		1-Naphthol's production and use in the production of agrochemicals, such as carbaryl, drugs, rubber oxidants and dye intermediates, may result in its release to the environment through various waste streams.
	Naphthalene	0.62		Naphthalene's production and use in the manufacture of phthalic anhydride, which is used as an intermediate in the production of phthalate plasticizers, resins, dyes, pharmaceuticals, insect repellents, and other materials may result in its release to the environment through various waste streams.
	Phenanthrene	0.11		Phenanthrene's production as a result of incomplete combustion of organic materials, will result in its release to the environment through various waste streams.
Alkyl-substituted aromatics	Sodium dodecylbenzenesulfonate	0.06		Sodium dodecylbenzenesulfonate's use as an anionic detergent results in its release to the environment through various waste streams.
	4-Octylphenol	0.1		This substance may be hazardous to the environment. Special attention should be given to fish.
Alicyclics	Cyclohexanone	48.2		Cyclohexanone may be released to the environment as a result of its manufacture, industrial use, transport, and disposal. Its major uses are as an intermediate in the production of adipic acid and caprolactam, which are used to make nylon 66 and nylon 6, respectively. It is also used as a solvent for materials such as synthetic resins, polymers, lacquers, inks, paint, and spot removers and a chemical intermediate in the production of plasticizers, insecticides, and other chemicals.

[a] Description of each chemical came from The Pubchem Progect (https://pubchem.ncbi.nlm.nih.gov/).

요약 (국문초록)

*Fusarium graminearum*의 cytochrome P450 효소들의 기능연구

신지영

붉은곰팡이(*Fusarium graminearum*)는 전 세계적으로 주곡작물인 벼, 보리, 밀, 옥수수 등에 큰 피해를 주는 식물병원균이다. 붉은곰팡이는 수확량의 감소 뿐 아니라 Trichothecene과 Zearalenone과 같은 곰팡이 독소를 생성하여 인축에 직접적인 피해를 유발한다. Cytochrome P450은 자연계의 거의 모든 생물 종에 존재하며, 약물, 발암물질, 독성물질, 스테로이드 등의 대사에 관여하는 물질이다. 현재 제약산업, 암 발생, 살충제 개발, 미생물을 이용한 생물산업, 환경산업 등의 다양한 분야에서 그 중요성이 대두되고 있다. 특히 cytochrome P450은 물질의 독성을 증가시키는 기능으로 인해 병 발생 원인으로 주목 받고 있다. 따라서 곰팡이 cytochrome P450에 대한 게놈수준에서의 접근이 필요하다는 인식이 대두되고 있지만, 식물병원균에서 전반적인 cytochrome P450의 기능 연구는 미흡한 실정이다. 붉은곰팡이에서 cytochrome

P450은 병원성에 관련되어 있는 Trichothecene 독소를 생합성 하는데 관여한다고 알려져 있고 곰팡이의 생존에 필요한 막 지질인 Ergosterol 생합성에 관여한다고 알려져 있다. 이번 연구에서 붉은곰팡이의 cytochrome P450의 기능적 분석을 위하여 생물정보학적 분석을 통해 119개의 P450 유전자를 동정하였고 이 중 102개의 유전자를 삭제한 돌연변이체를 확보하였다. 102개의 유전자 삭제 돌연변이체를 병원성 실험, 외래물질 분해 등 38개의 조건에 서 관찰 하여 phenotypic dataset를 구축하였다. 이번 연구에서 구축된 붉은곰팡이의 cytochrome P450 mutant library는 붉은곰팡이의 대사 물질 연구(metabolomics) 분야 개척의 발판이 될 수 있을 것이다.

주요어: 붉은곰팡이, cytochrome P450 (사이토크롬 피450), 병원성, 외래물질 분해, phenotypic dataset (표현형 데이터 모음), mutant library

학번: 2012-233383